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Regeneration of Melanomas in Fishes[1]

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(Plates I-IV; Text-figures 1-45)

COMPARATIVE pathologists who have studied the black pigment cell tumors of sharks, fishes, amphibians, reptiles, birds and mammals, have discovered that the melanocyte is the common cell type of the melanomas of vertebrate animals, including those of man (Gordon, 1951b). The living melanocytes from the melanomas of man, mouse and fish, when studied by tissue culture methods, have been shown to be similar morphologically (Grand, Gordon & Cameron, 1941, and Attardi & Moro, 1953). A comparative study of the enzyme reactions of melanomatous tissues from mammals and fishes also revealed that both are quite similar with respect to tyrosinase activity (Fitzpatrick, Lerner, Calkins & Summerson, 1950).

An outstanding peculiarity of the fish melanoma, in contrast with human melanoma, is the presence of macromelanophores. The large size of these melanophores and their capacity for drastic changes in the shape of their pseudopodial processes precludes their being confused with the much smaller melanocytes or the melanin-laden macrophages. From previous studies it was not possible to state whether macromelanophores and melanocytes were related ontogenetically, but it was known that melanomas arose in hybrid fishes only when macromelanophores were present.

Regeneration studies make possible the retracing of the progressive growth stages in the development of a melanoma. It will be shown that, in part, they confirm the observations of Gordon & Smith (1938) who demonstrated that in the earliest stages of this neoplasm, macromelanophores in their abnormal growth replace the normal tissues of the corium almost completely and create a state of melanosis. In later stages, Gordon & Smith showed that the large black pigment cells invade the deeper body areas, moving along the fascial tissues of the muscles. The macromelanophores eventually surround the muscle fibres and destroy them. Nodular lesions leading to the formation of melanomas usually arise in these primary zones of diffuse melanosis. During the early stages in the growth of the melanoma, the macromelanophores are the principal cells involved. As the growth of the melanoma progresses, the macromelanophores apparently redifferentiate into cells which have the morphological properties of melanocytes.

Study of these suspected cellular transformations of normal macromelanophores to malignant melanocytes, made possible by histological examination of the progressive growth of melanomas, obviously required additional study by more direct methods. Observations of regenerating tissues following the amputation of melanomas in the dorsal fin have made it possible to retrace the ontogenetic relationships between macromelanophores and melanocytes. By experiments that will be described, the close association of these pigment-forming cells has been confirmed. In an independent study, Marcus & Gordon (1954) followed the fate of melanoma fragments after they were transplanted into clear, transparent normal areas of the fish's skin. They, too, found that some melanocytes are capable of transforming into macromelanophores and vice versa.

Material and Methods

Fifty-three xiphophorin fishes were studied; they consisted of young and adult platyfish, *Xiphophorus maculatus*, and platyfish-swordtail

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hybrids, *X. maculatus–X. helleri*. Their histories are listed in Table 1.

The fishes chosen for the study of the effects of amputation of normally and atypically pigmented dorsal fins were obtained from genetically known stocks maintained at the Genetics Laboratory of the Aquarium, New York Zoological Society, by methods described by Gordon (1950a). The most important pigmentary patterns for these studies were those that are produced in the dorsal fins by two types of specialized pigment-forming cells, the micromelanophores and macromelanophores. Various combinations of these two kinds of pigment effector cells in the dorsal fins of fishes were available, so that it was possible to compare the results of amputation and regeneration of normally pigmented dorsal fins with those that were either in a state of melanosis or had melanomas. In those that had melanomas, it was also possible to compare those that had the typical black coloring with those that had amelanotic melanomas.

The fishes were first anesthetized with a 1:2000 solution of MS 222 (Triacine Methanesulfonate produced by Sandoz) and placed on a wet cotton pad. The dorsal fins were removed close to the body, using iridectomy scissors. The regenerating fins were observed at intervals under the binocular microscope and drawings were made of the progressive regrowths.

For histological study, tissues amputated and regenerated were fixed in Bouin's solution, decalcified in nitric acid-phloroglucin, dehydrated in dioxane, embedded in paraffin and sectioned at 8 to 10 micra. Sections were studied either with Mayer's hematoxylin (hemalum) and eosin, or with a modification of the Masson's trichrome stain. In order to obtain the cellular details of extremely black tumors, the sections were first treated with celloidin and then bleached in a solution of potassium chlorate crystals in hydrochloric acid and 70% alcohol.

Regeneration of Dorsal Fins in Platyfish

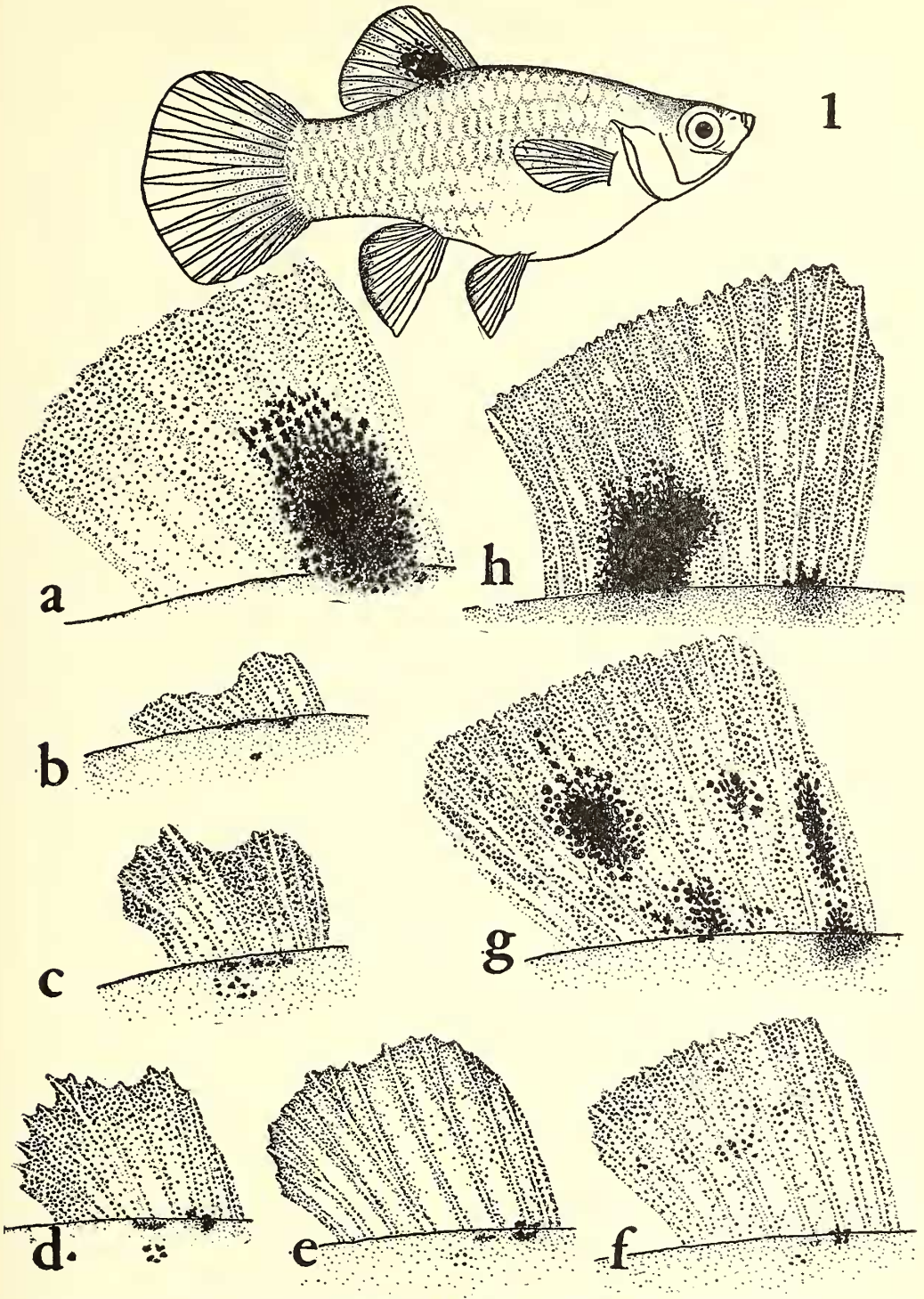
The spotted dorsal fins of two female and three male platyfish were amputated, Table 1, No. 1. Each fin had several groups of normal macromelanophores as well as many micromelanophores, Text-fig. 1. Within 24 hours wound healing began and within five to seven days the blastema, about 0.5 mm. in height, was formed. The blastema contained some pigment granules and the stumps of the fin rays, each of which was composed of two lepidotrichial elements. Within two weeks the blastema grew to about 1 to 1.5 mm. in height. Micromelanophores appeared along the regenerating fin rays, in the fin membranes and on the fin's distal edge, Text-fig. 1b. Within one to one and a half months the fin grew to 2 to 3 mm., Text-figs. 1c, 1d. In two animals some macromelanophores migrated into the base of the regenerating fin from the region ventral to the dorsal fin, Text-fig. 1d. The fin rays began to bifurcate at their tips. At three to four months in four out of five fish, the new dorsal fin reached its original size, 5 to 6 mm. in height. Only eight fin rays regenerated in one fish to replace the original 10, Text-fig. 1f. In one animal originally having several spots on its dorsal, within eight months, the macromelanophores formed one big spot at the posterior base of the regenerated fin, Text-figs. 1g, 1h. In another fish the large spots did not form at all. Usually macromelanophores appeared in the

Table 1. Genetic Histories of Fishes Used in Regeneration Studies of Normal and Atypical Pigment Cell Growths in the Dorsal Fins[1]

Group	No.	Pedigree	Genetic Constitution	Melanophores
1.	5	163	*Sd* + + Spotted-dorsal	Micro. + Macro.
2.	2	306	+ *Co* + Comet	Micro.
3.	3	306	+ *Co E* Wagtail	Micro.
4.	5	306	*Sd Co* + Spotted-dorsal, Comet	Micro. + Macro.
5.	3	306	*Sd Co E* Spotted-dorsal, Wagtail	Micro. + Macro.
6.	2	311, 316	+ + Unpatterned	Micro.
7.	18	311, 316	*Sd* + Melanosis, variable (1, 2, 3)[2]	Micro. + Macro.
8.	10	311, 316	*Sd* + Melanomas	Micro. + Macro.
9.	5	311, 316	*Sd i* Amelanotic melanoma	Not evident

[1]Summary of 53 histories. Groups 1 to 5 represent *Xiphophorus maculatus*, 6 to 9 represent *X. maculatus–X. helleri* hybrids. The individual records are presented in Tables 2 to 6.

[2]The degree of melanosis is indicated arbitrarily: 1, severe; 2, intermediate; 3, slight; see text for criteria.



TEXT-FIG. 1. Regeneration of the spotted dorsal fin of a platyfish, genetically *Sd co e*. Macromelanophores produce the spotted pattern in the dorsal fin. Micromelanophores are also present in the fins and on the body. 10 ×. **a**—Before amputation. **b**—Within 13 days following amputation. **c**—Within 40 days. **d**—Within 63 days. **e**—Within 90 days. **f**—Within 120 days. **g**—Dorsal fin of another fish of the same stock before amputation. **h**—The regenerate of the fin shown in figure g within 8 months following amputation.

Table 2. Regeneration, after Amputation, of Dorsal Fins and the Spotted Patterns in Platyfish[1]

No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Pigmentation
1.	Spotted-dorsal	240	Complete	Similar
2.	Spotted-dorsal	240	Complete	Lacking
3.	Spotted-dorsal	229	Complete	Lacking
4.	Spotted-dorsal	188	Complete	Lacking
5.	Spotted-dorsal	62	Complete	Lacking

[1]*Xiphophorus maculatus* of pedigree number 163, see Table 1, item 1, for genetic constitution.

regenerated tissues, if at all, much later than the micromelanophores.

The dorsal fins of two comet platyfish with micromelanophores only, Table 1, No. 2, were amputated for purpose of comparison. The comet pattern is made up of closely grouped micromelanophores and is confined to the caudal fin only, Text-fig. 2. Within a week a blastema developed, 0.3 to 0.5 mm. in height, which contained fin rays and some micromelanophores at the base, Text-fig. 2b. The number of micromelanophores increased along the fin rays and they covered the base of the fin, Text-fig. 2c; they reached the distal edge of the new fin in two weeks. Within a month, the height of the regenerate was 3.5 to 4 mm., and some of the regenerating fin rays had bifurcated, Text-fig. 2d. Within two to three months the new fin reached its original height, 5 to 6 mm., and pigmentary pattern, Text-fig. 2e. Within four to seven months there were no further appreciable changes except for the secondary bifurcation of some fin rays.

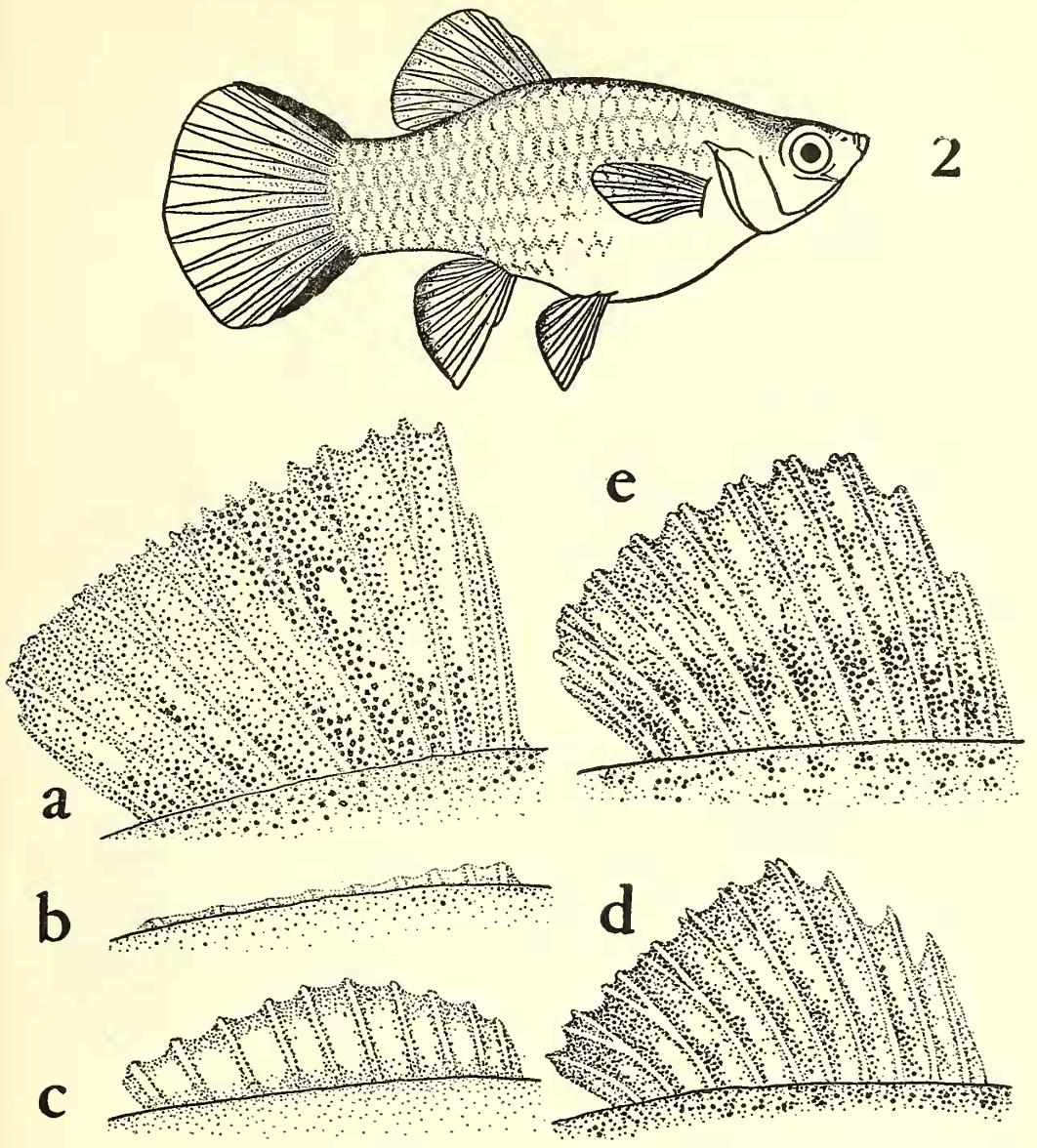
The dorsal fins of three platyfish with the wagtail pattern were amputated next, Table 1, No. 3. The dorsal fins of the wagtail platyfish had micromelanophores only, but they were much more numerous in all the fins than in the fins of the comet platyfish. The fins of the wagtail regenerated normally; however, the rate of the micromelanophore restoration and the number of regenerating micromelanophores were greater than in the comet platyfish.

The heavily spotted dorsal fins of five platyfish were amputated; they had both micro- and macromelanophores in the dorsal fin and a comet pattern in the tail, Table 1, No. 4. One specimen had macromelanophores that had spread down from the dorsal fin to both sides of the body. There they had infiltrated the underlying tissues, creating a condition of melanosis. Within a week following amputation of the dorsal fins, blas-

Table 3. Regeneration, after Amputation, of Dorsal Fins and Pigmentary Patterns in Platyfish[1]

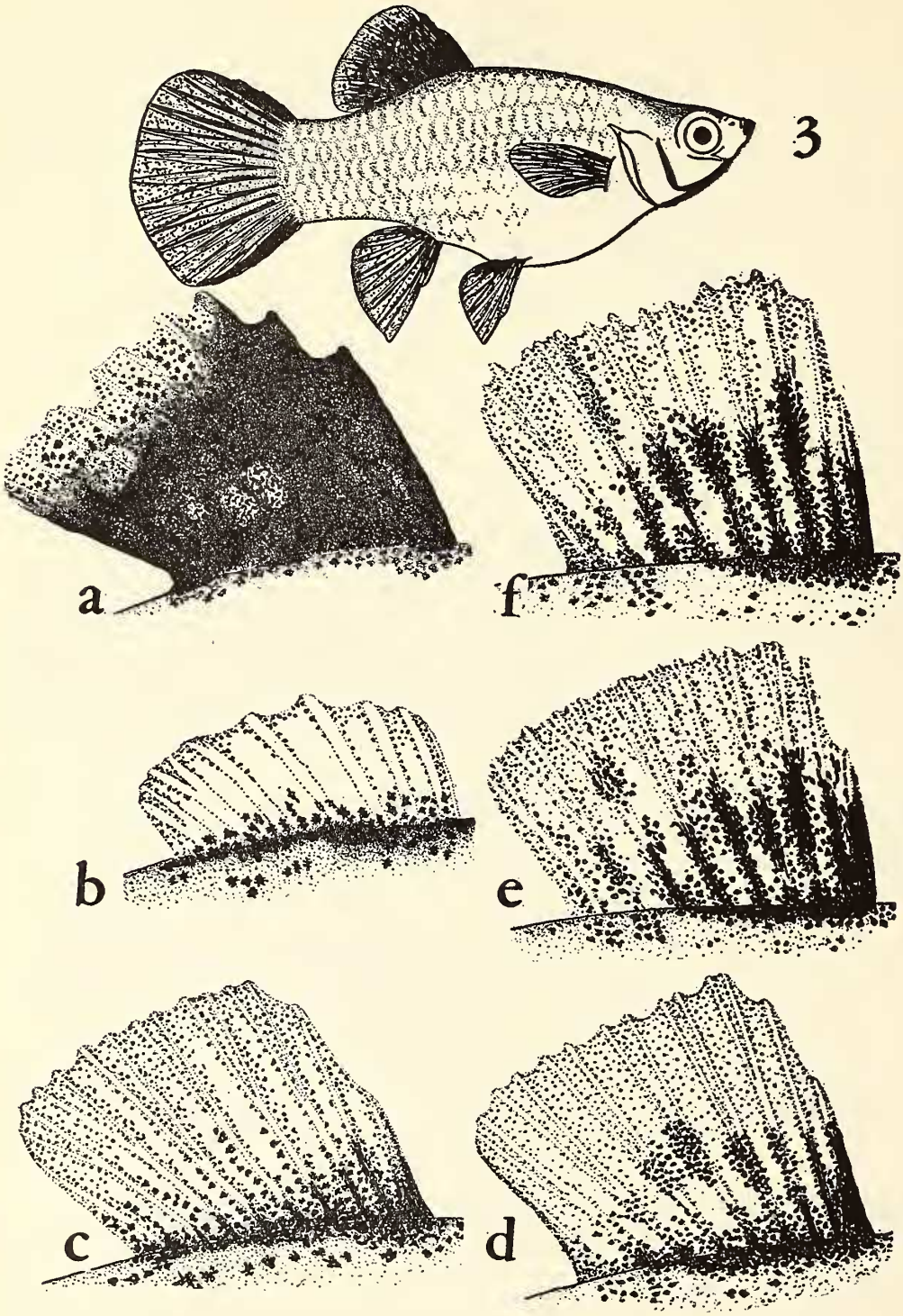
No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Pigmentation
6.	Comet[2]	233	Complete	Complete
7.	Without Comet	23	Incomplete	Fixed[3]
8.	Wagtail[2]	190	Complete	Complete
9.	Wagtail[2]	7	Incomplete	Fixed[3]
10.	Wagtail[2]	2	Incomplete	Fixed[3]
11.	Spotted-dorsal, Comet	180	Complete	Incomplete
12.	Spotted-dorsal, Comet	90	Incomplete	Incomplete
13.	Spotted-dorsal, Comet	1	Incomplete	Fixed[3]
14.	Spotted-dorsal, Comet	21	Incomplete	Incomplete
15.	Spotted-dorsal, Comet	15	Incomplete	Fixed[3]
16.	Spotted-dorsal, Wagtail	180	Complete	Incomplete
17.	Spotted-dorsal, Wagtail	104	Complete	Incomplete
18.	Spotted-dorsal, Wagtail	55	Complete	Incomplete

[1]*Xiphophorus maculatus* of pedigree number 306, see Table 1, items 2, 3, 4, 5.
[2]Comet and Wagtail patterns are made up of micromelanophores only.
[3]Sacrificed for histological study.



TEXT-FIG. 2. Regeneration of the unspotted dorsal fin in a comet platyfish, genetically *sd Co e*. No macromelanophores are present in this specimen. 10 ×. **a**—Before amputation. **b**—Within 1 week following amputation. **c**—Within 2 weeks. **d**—Within 30 days. **e**—Within 60 days.

temas developed that were 0.3 to 0.5 mm. and contained some pigment particles and fin ray stumps. Within two weeks the blastemas were 0.5 to 1 mm. in height and contained many micromelanophores and four to five macromelanophores that had migrated from the base of the fin. Within two to three months the new fins of four of the platyfish were as high as the originals; one of them regenerated in slightly more than three months. Within two months the macromelanophores in the fins reformed the typical spotted dorsal pattern. Almost no micromelanophores were found in the spotted region, whereas many of them were present in the distal region of the fin. As time went on, more macromelanophores continued to migrate into the new fins, increasing the size of the spots, but even at six months the size of the macromelanophore spottings in the dorsal fin was not as large as in the originals.



TEXT-FIG. 3. Regeneration of spotted dorsal fin of a wagtail platyfish, genetically *Sd Co E.* This type of platyfish has many more micromelanophores in its fins than the *Sd co e* fish shown in Text-figure 1. 10 ×. **a**—Before amputation. **b**—Within 10 days following amputation. **c**—Within 33 days. **d**—Within 62 days. **e**—Within 120 days. **f**—Within 150 days.

The heavily spotted dorsal fins of three wagtail platyfish, Table 1, No. 5, were amputated. A strong melanosis produced by both micro- and macromelanophores existed in the dorsal fins Text-fig. 3a. Within a week a blastema formed, 0.5 to 1 mm. in height, which contained pigment particles and stumps of the fin rays. After 10 days the fin rays had begun to regenerate in the normal way and the micromelanophores reached the distal edge of the fin, producing a "Pigmentsaum" effect, a term used by Bösenberg (1938); during the same period macromelanophores also migrated into the fin from its base, Text-fig. 3b. After two weeks the regenerated fin was 1.5 mm. in height. Within a month it was 3 to 4 mm. and the micro- and macromelanophores had increased in number and the latter had begun to form black spots at the base of the fin, Text-fig. 3c. The macromelanophores had migrated over and along the fin rays; as a consequence, the basal part of the rays was completely covered. Within two to three months the new fin attained its previous size, 4.5 to 5 mm. in height. The macromelanophores continued to invade the regenerated fin, Text-figs. 3d to f. But even six months after the amputation the melanosis was not as strong as it was in the original fin, Text-fig. 3g. It appears, then, that after the fins regenerate normally they are invaded by macromelanophores which produce a somewhat lesser state of melanosis.

Regeneration of the Dorsal Fins in Species Hybrids between the Platyfish and Swordtail

Some spotted-dorsal hybrids exhibited melanosis, others typical melanomas, while still others showed amelanotic melanomas in their dorsal fins. For purposes of comparison, however, two platyfish-swordtail hybrids without macromelanophores but with micromelanophores in the dorsal fins were first studied, Table 1, No. 6. Within one week after amputation, blastemas 0.2 to 0.5 mm. were formed. Within two weeks the blastemas were 1 to 1.5 mm. and contained fin rays. Micromelanophores soon reached the distal edge of the regenerating fins, forming pigmented borders. The pigment cells migrated along both sides of the fin rays, leaving clear areas between the rays. Within two months the fin rays began to bifurcate. Within three months the fins reached their previous size of 5 to 6 mm.; their former pigmentary patterns were at that time restored.

The regeneration process was observed in eighteen platyfish-swordtail hybrids with dorsal fin melanosis, Table 1, No. 7. In some hybrid fish the melanosis was strong; in others just a few discrete macromelanophores were present in the dorsal fins. Most hybrids had, in addition to a melanosis of the dorsal fin, a melanosis of the tail and body, especially in the region under the dorsal fin, Text-fig. 4.

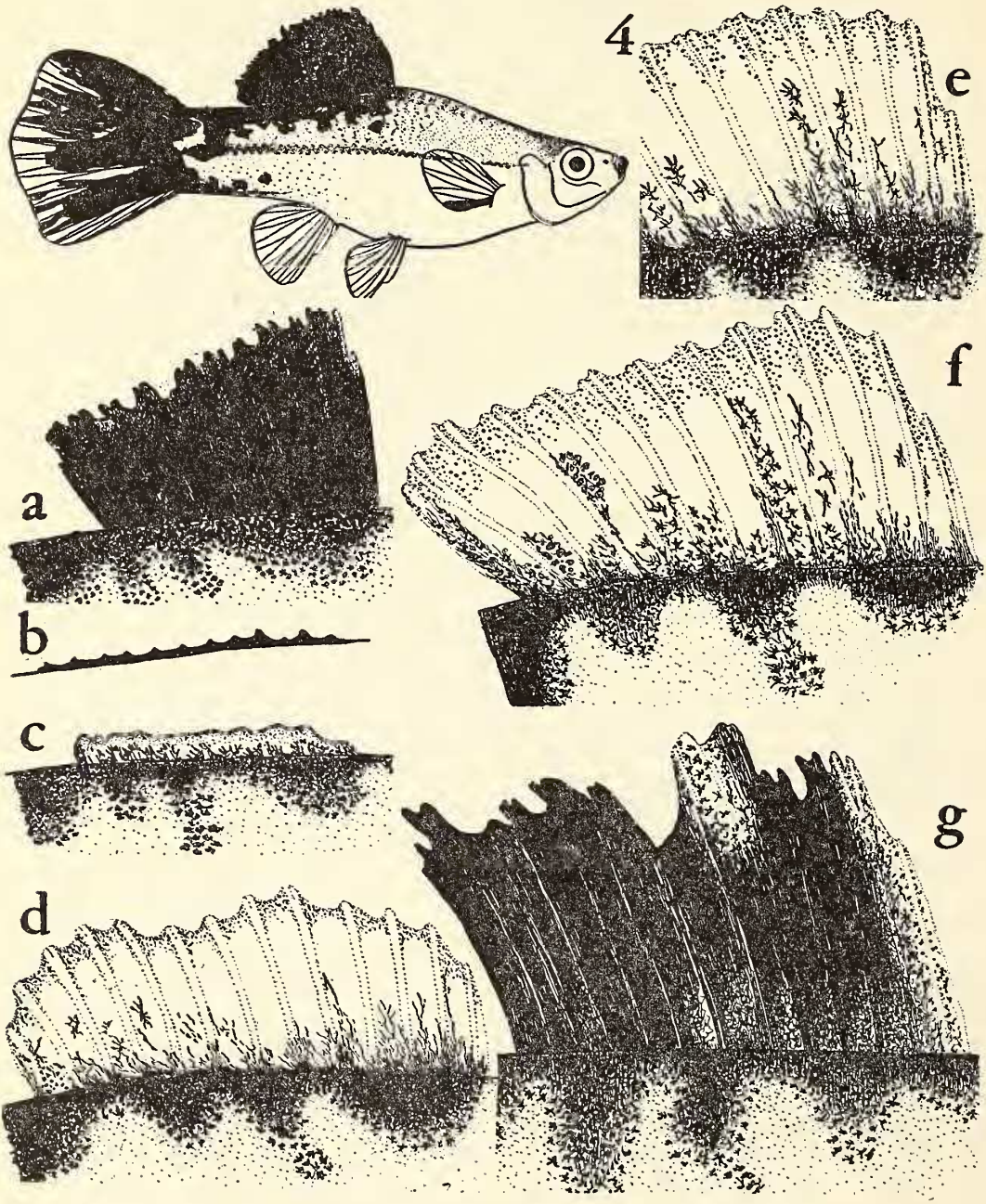
After amputation of the dorsal fins of fish with incomplete melanosis, blastemas containing some pigment particles were formed within five to seven days. Within 10 to 15 days the regenerated fins were 1 to 1.5 mm. and contained micromelanophores and fin rays. Macromelanophores began to appear at the base of the regenerating fins within 14 days in those that had been highly melanotic, and within 25 to 30 days in others. Within about three months the regenerated fins reached their original size, 8 to 10 mm. The new pigment patterns produced by the macromelanophores in the dorsal fins were not always the same, nor was the degree of melanosis as great as in the originals.

In those fish showing strong melanosis in their dorsal fins, the regeneration of the macromelanophores was more rapid; one, Text-figs. 4 to 4g, will be described in detail. During the healing of the wound, the epidermis contained dense pigment particles derived from the degenerating melanophores; this cleared up within one week. Dendritic processes of the macromelanophores, located just below the removed fin, penetrated the blastema and later whole cells entered it, Text-figs. 4b, 4c. Within two weeks the regenerated fin was 3 mm. and had many micromelanophores in its distal part; discrete macromelanophores appeared between the rays and formed a melanosis at the base of the fin, Text-fig. 4d. The fin reached its full height within two months, Text-figs. 4e to 4g, during which time melanosis was intensified. The 8½-months-old regenerated fin was almost as melanotic as the original fin, Text-fig. 4g.

The migration paths of the macromelanophores from their prior positions on the body just ventral to the fin into the regenerated fin between rays 7 and 8 may be seen in Text-fig. 4h. Distally some discrete macromelanophores contain fine grayish melanin granules that represent newly formed macromelanophores; these have been transformed from melanocytes. Within two months the previously produced darker macromelanophores that had originated from the base of the fin, and the newly formed gray macromelanophores, came together and produced a state of melanosis, Text-figs. 4i, 4j. Thus, there are two sources of macromelanophores in the regenerate: from those that were already present below the fin and from macromelanophores that are formed *in situ* from melanocytes in the regenerate itself.

The development of melanosis was faster in young hybrids than in mature ones. For ex-

ample, in young animals of 16 to 20 mm. in standard length with complete melanosis in the dorsal fin, as well as in the body below the dorsal fin, the macromelanophores migrated into the blastema within four days after amputation. Within three weeks the large black pigment cells reproduced an almost complete melanosis. The regeneration of the dorsal fin of one young fish of 21 mm. in length was peculiar. The anterior part of its original fin and the

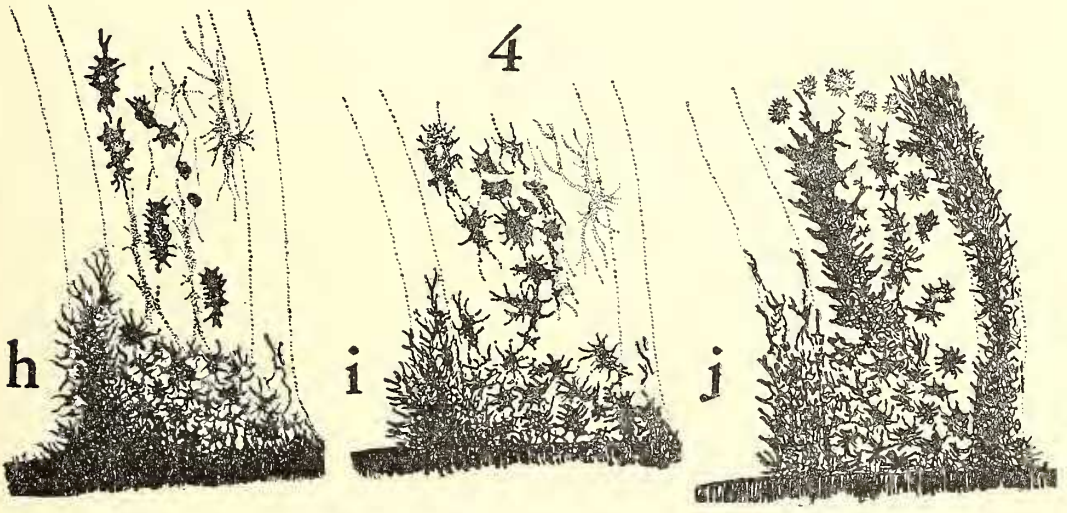


TEXT-FIG. 4. Regeneration of the spotted dorsal fin of a platyfish-swordtail hybrid with severe melanosis in the dorsal fin. a to g, 5×. **a**—Before amputation. **b**—Within 2 days after amputation. **c**—Within 7 days. **d**—Within 14 days. **e**—Within 21 days. **f**—Within 30 days. **g**—Within 250 days; note that melanosis is almost the same as it was in the uncut fin, figure a.

TABLE 4. REGENERATION, AFTER AMPUTATION, OF DORSAL FINS AND PIGMENTATION IN PLATYFISH-SWORDTAIL HYBRIDS[1]

No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Pigmentation
19.	Micromelanophores[2]	165	Complete	Complete
20.	Micromelanophores[2]	120	Complete	Complete
21.	Melanosis (3)[3]	257	Complete	Melanosis (2)
22.	Melanosis (2)	196	Complete	Melanosis (1)
23.	Melanosis (2)	180	Complete	Melanosis (2)
24.	Melanosis (2)	25	Incomplete	Fixed
25y.[4]	Melanosis (3)	30	Incomplete	Melanosis (3)
26.	Melanosis (2)	1	Incomplete	Fixed
27.	Melanosis (3)	1	Incomplete	Fixed
28y.	Melanosis (3)	21	Complete	Melanosis (3)
29y.	Melanosis (3)	6	Incomplete	Fixed
30.	Melanosis (3)	27	Incomplete	Fixed
31.	Melanosis (3)	30	Incomplete	Melanosis (2)
32.	Melanosis (3)	4	Incomplete	Fixed
33.	Melanosis (3)	210	Complete	Melanosis (3)
34y.	Melanosis (2)	30	Bilobed	Melanosis (1)
35y.	Melanosis (2)	30	Complete	Melanosis (1)
36.	Melanosis (2)	30	Incomplete	Melanosis (1)
37.	Melanosis (2)	9	Incomplete	Fixed
38.	Melanosis (2)	280	Complete	Melanosis (1)

[1]*Xiphophorus maculatus–Xiphophorus helleri* hybrids, pedigree numbers 311 and 316, see Table 1, items 6, 7.
[2]Fish numbered 19 and 20 had no macromelanophore patterns in dorsal fins; used for controls.
[3]The severity of melanosis is indicated by numbers in parentheses.
[4]y represents an immature specimen.



TEXT-FIG. 4 (*Continued*). h–The proximal area of the regenerate between 7th and 8th fin rays as seen under higher magnification, after 30 days. The macromelanophores are the dark cells which had moved up from the base of the fin. The melanocytes are the gray cells. These contain fine, dispersed melanin granules. i–Same after 38 days. Newly formed macromelanophores and melanocytes are located between the rays. j–After 60 days. The growth of macromelanophores has created a state of melanosis.

Table 5. Regeneration, after Amputation, of Melanomas in the Dorsal Fins of Platyfish-Swordtail Hybrids[1]

No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Melanoma
39.	Melanoma on and below fin	75	Bilobed	Melanoma
40.	Melanoma	300	Bilobed	Melanoma
41.	Melanoma	180	Bilobed	Melanosis (3)
42.	Melanoma	346	Complete	Melanoma
43.	Melanoma	69	Bilobed	Melanosis (3)
44.	Melanoma on and below fin	46	Complete	Melanosis (3)
45.	Melanoma on and below fin	1	Incomplete	Fixed
46.	Melanoma on and below fin	20	Incomplete	Fixed
47.	Melanoma	198	Complete	Melanosis (3)
48.	Melanoma	13	Incomplete	Fixed

[1] *Xiphophorus maculatus-helleri* hybrids of pedigree number 311, see Table 1, item 8.

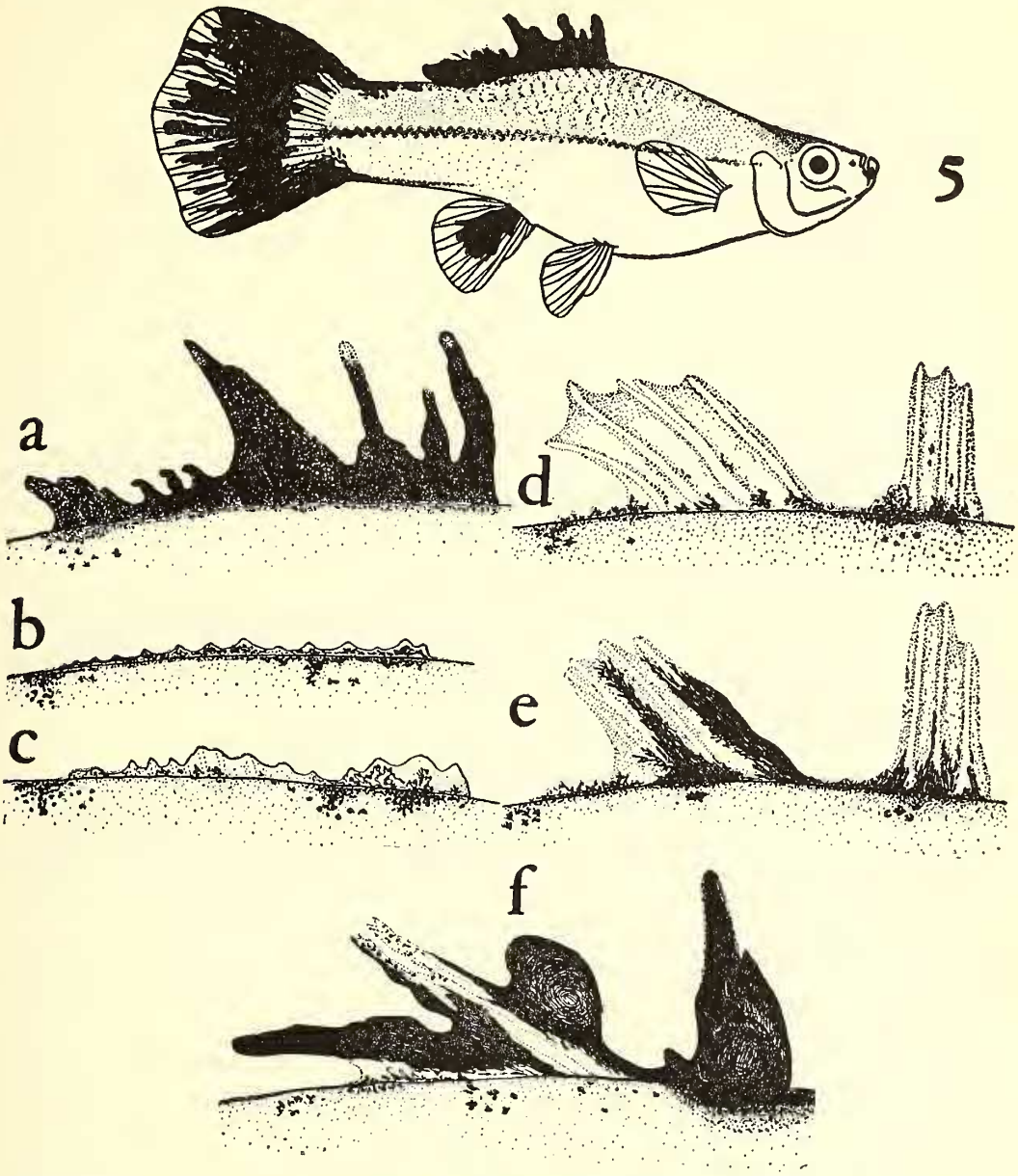
body just below it were entirely melanotic, whereas the posterior part of the fin and the body below it were not. After amputation the middle part of the fin did not regenerate. Within a month the anterior part of the new fin grew to 3 mm. and showed an almost complete melanosis, whereas the posterior part, which was 2 mm., showed a much lesser degree of melanosis. These observations suggest that when the tissues underlying the removed fin are completely melanotic at the time of operation, the melanosis in the regenerated fin develops more rapidly and becomes complete.

The regenerations of fins in 10 platyfish-swordtail hybrids with melanotic melanomas in their dorsal fins were observed, Table 1, No. 8. In some of these hybrids the dorsal fins were destroyed to various degrees, and in others secondary melanomas had developed on various parts of their bodies, Text-figs. 5 and 6.

In hybrids that had melanomas in the dorsal fin only, the regenerated fins were usually abnormal. Within a week after a melanomatous fin was amputated, a blastema formed which contained much pigment cell debris, Text-figs. 5a, 5b. Within one month the anterior and posterior parts of the fin regenerated but not the middle, Text-figs. 5c, 5d. After two months the regenerated fin became melanotic, Text-fig. 5e. Within 10 months a melanoma formed at the base of the anterior part of the fin, and a second nodular melanoma developed on the posterior part, Text-fig. 5f. In another animal, within six months following amputation, the regenerated fin was in a state of melanosis; it was then fixed for the histological study. After amputation of a destructive, bilobed, melanotic melanoma in the dorsal fin of a third hybrid, a non-tumorous, bilobal, deeply pigmented fin regenerated within 11 months. It measured 7.5 mm., whereas the removed fin had been only 1.5 mm. posteriorly and 5.5 mm. anteriorly.

In hybrids that had melanoma both in the dorsal fin and on the body ventral to it, the redevelopment of tumors in the regenerated fins was more rapid. For instance, one hybrid had a melanoma of the dorsal fin as large as the fin itself, and had infiltrated the tissue just below the fin, Text-fig. 6. After the fin together with the growth had been amputated, a melanotic bilobed fin developed at first, Text-figs. 6b to 6e, and then after 75 days a melanoma developed at the base of the anterior lobe and another around the posterior part, Text-fig. 6f. These observations suggest that when the melanoma is restricted only to the dorsal fin, the period of redevelopment of the tumor in the regenerated fin is longer than in those fish that exhibit melanoma both on the dorsal fin and on the body below that fin.

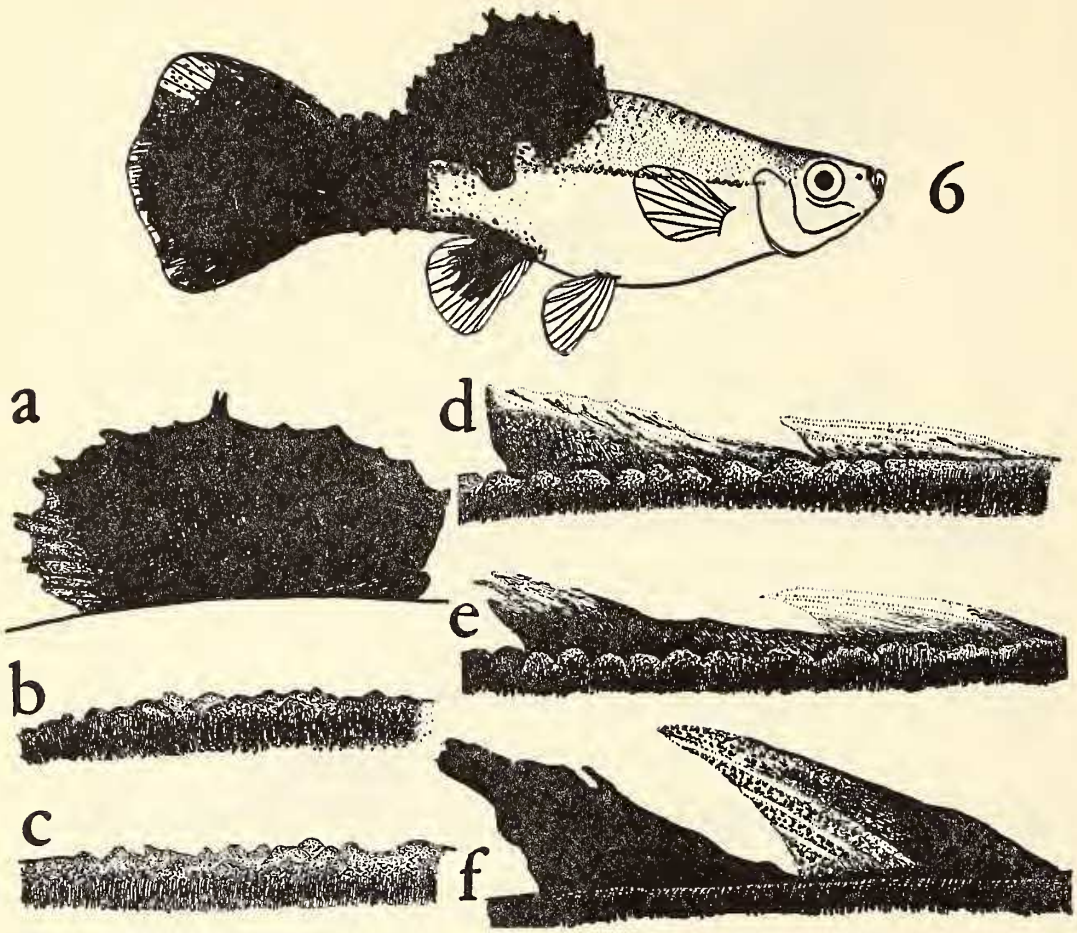
The fins of five platyfish-swordtail albino hybrids that had *amelanotic melanomas* in their dorsal fins were amputated, Table 1, No. 9. The melanomas were heavy and pink-colored; they had partly invaded the bodies below the dorsal fins. Each fish that had its melanomatous dorsal fin amputated reacted differently, but the responses were only slightly different from those



TEXT-FIG. 5. Regeneration of melanomas in the dorsal fin of a platyfish-swordtail hybrid. 5×. **a**—Before amputation. **b**—Within 7 days following amputation. **c**—Within 14 days. **d**—Within 30 days. **e**—Within 60 days. **f**—Within 10 months.

of the fish having typical melanomas that were previously described.

In one albino hybrid the original tumor was nodular and restricted to the anterior part of the fin. This part of the fin was either surrounded or destroyed by the tumor; the posterior part was also tumorous but the fin rays were still visible. The entire tumor of the dorsal fin contained large branching blood vessels clearly visible near its surface. Within a week following amputation, a 0.6 mm. blastema formed which contained no visible blood vessels or fin rays. At two weeks the regenerated fin measured 1 mm.; within one month it was 2 mm. and had five visible fin rays on its distal edge. Apparently the blastema and the tumor developed simultaneously, because within two months the basal one-third of the regenerated fin had a heavy melanoma; the re-

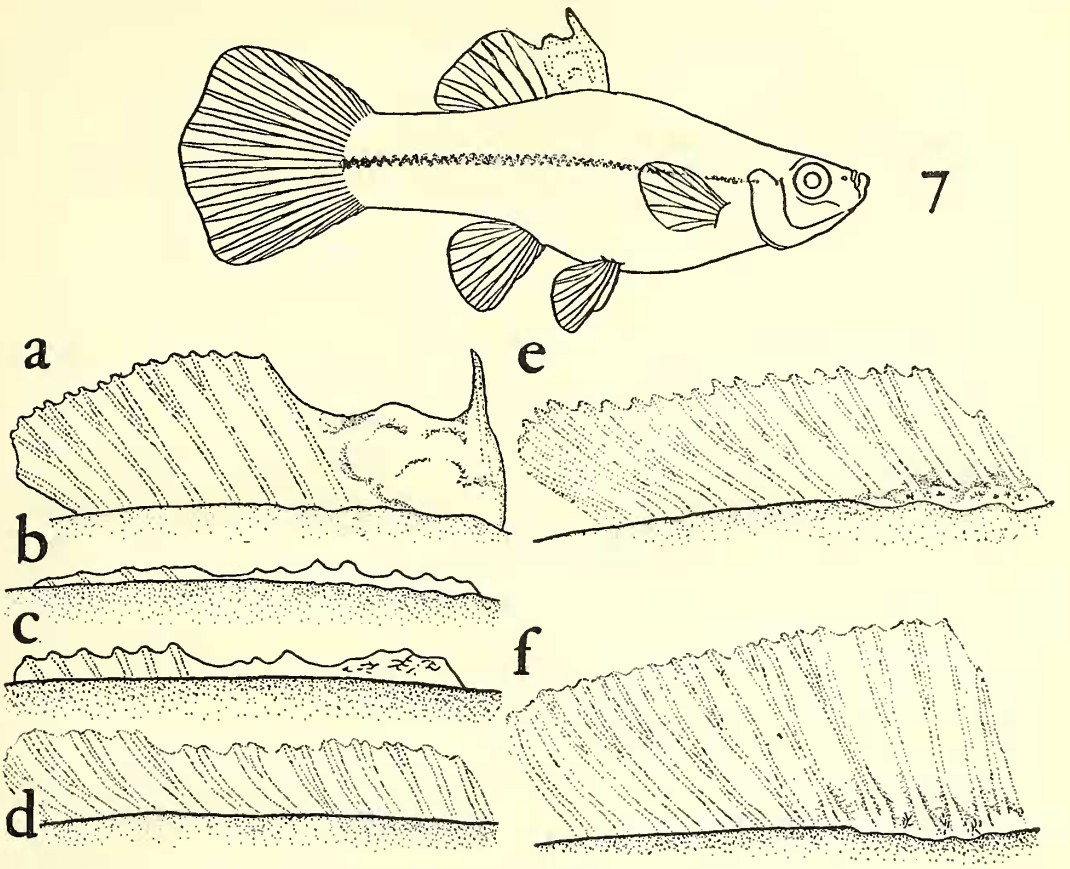


TEXT-FIG. 6. Regeneration of a melanoma in the dorsal fin of a platyfish-swordtail hybrid in which the melanoma had extended into the body ventral to the fin. 10 ×. **a**—Before amputation. **b**—Within 7 days following amputation. **c**—Within 14 days. **d**—Within 21 days. **e**—Within 30 days. **f**—Within 45 days.

TABLE 6. REGENERATION, AFTER AMPUTATION, OF AMELANOTIC MELANOMAS IN THE DORSAL FINS OF PLATYFISH-SWORDTAIL HYBRIDS[1]

No.	Pattern	In days	Regeneration Of Fin	Regeneration Of Melanoma
49.	Amelanotic melanoma	90	Complete	Amelanotic melanoma at base of fin
50.	Amelanotic melanoma on, below fin	90	Complete	Amelanotic melanoma
51.	Amelanotic melanoma on, below fin	60	Complete	Amelanotic melanoma
52.	Amelanotic melanoma on, below fin	20	Incomplete	Fixed
53.	Amelanotic melanoma on, below fin	30	Incomplete	Amelanotic melanoma

[1]*Xiphophorus maculatus-helleri* hybrids of pedigree 311, see Table 1, item 9.



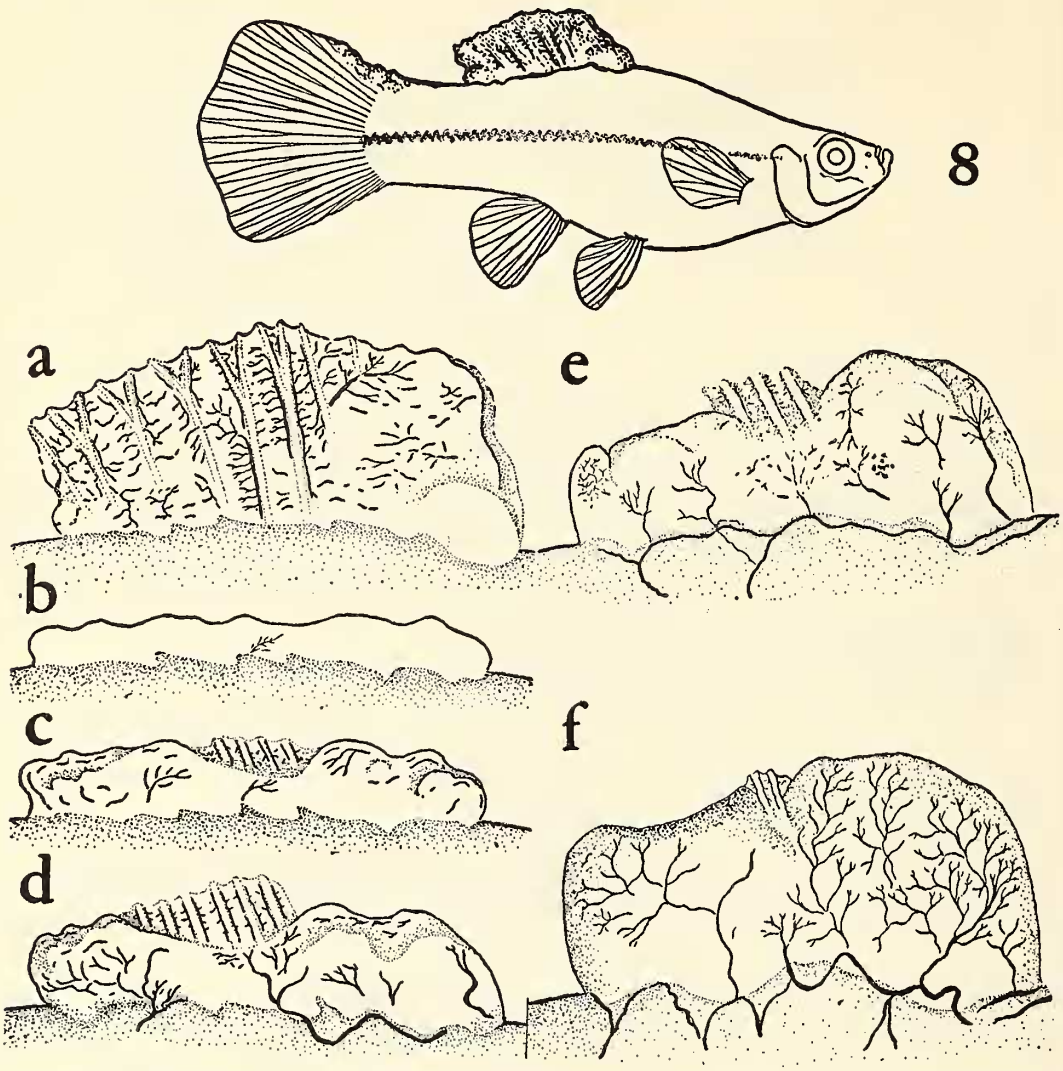
TEXT-FIG. 7. Regeneration of an amelanotic melanoma in the dorsal fin of a platyfish-swordtail hybrid. 6 ×. **a**—Before amputation. **b**—Within 1 week following amputation. **c**—Within 17 days. **d**—Within 1 month. **e**—Within 2 months. **f**—Within 3 months.

mainder was normal and contained 12 rays. All parts of the new growth were highly vascularized, but its general appearance was entirely different from that of the original fin. The fish died two months after its fin was amputated.

In another hybrid, an amelanotic melanoma in the anterior region of a 10-rayed dorsal fin was amputated, Text-fig. 7. A blastema containing four fin ray stumps developed within a week, Text-fig. 7b. Within two weeks the regenerated fin grew to 1 mm. and contained six fin rays, Text-fig. 7c. Within a month it was 3 mm. and had 13 rays, three more than the original one, Text-fig. 7d. A melanoma had developed at its former site, at the base of the anterior region of the fin, Text-fig. 7e. Within three months the regenerated fin reached its previous height of 5 mm., at which time the fish was fixed for histological study.

Another albino had a heavy, highly vascularized melanoma in its dorsal fin in which some fin rays were visible, particularly their tips. The tumor had infiltrated the body below the fin and a secondary melanoma was present on the dorsal edge of the tail, Text-fig. 8. Within a week after its amputation a blastema developed into an amorphous mass about 1 mm., Text-fig. 8b; within 17 days it was 2 mm., more vascularized, and contained four fin rays, Text-fig. 8c. Within one month the regenerating fin had six rays and a larger melanoma, Text-fig. 8d. At two months the new growth measured 4 mm.; it had destroyed all but three of the regenerated rays, Text-fig. 8e, and these were reduced to two within three months. During these three months the highly vascularized amelanotic melanoma reached its original size of 5.5 mm., Text-fig. 8f. The fish was then fixed for histological study.

The dorsal fin of another albino hybrid with an amelanotic melanoma measuring 1.5 mm. grew back after amputation to 4 mm., a size considerably larger than that of the original fin. The regrowth of the fin is thus not necessarily impeded by the simultaneous growth of a melanoma.



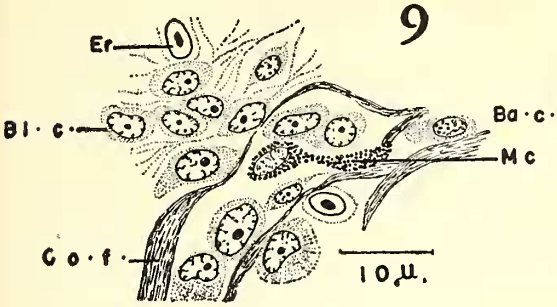
TEXT-FIG. 8. Regeneration of a large amelanotic melanoma in the dorsal fin of a platyfish-swordtail hybrid. 6 ×. **a**—Before amputation. **b**—Within 1 week following amputation. **c**—Within 17 days. **d**—Within 1 month. **e**—Within 2 months. **f**—Within 3 months.

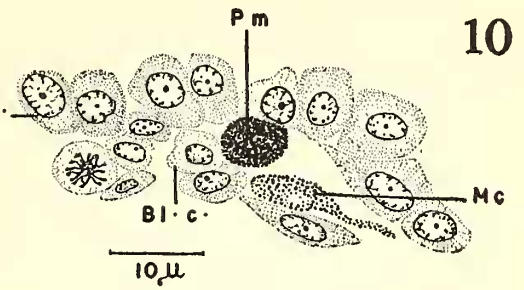
HISTOLOGICAL OBSERVATIONS OF REGENERATED DORSAL FINS

Histological observations of the regeneration process of the normal dorsal fins of platyfish and platyfish-swordtail hybrids having micromelanophores only (represented in Table 1, Nos. 2, 3 and 6) may serve as a basis for comparison with the regeneration process in fish having abnormally pigmented fins (represented in Table 1, Nos. 7, 8 and 9).

Within 12 hours after amputation of the normally pigmented fins, macrophages phagocytize melanin particles and other cell debris. The squamous cells of the epithelium moving from both sides of the wound grow over its surface to reform the epidermis. In the epidermis, the epithelial cells are fibrillar and loosely arranged; the macrophages that contain melanin particles are round or oval and have eccentric nuclei.

Within two or three days the basal cells of the adjacent epidermis move under the squamous epithelial cell layer. Collagenous fibres become more abundant in the connective tissues below the regenerating epidermis. Between these fibres are scattered fibroblasts, lymphocytes, granulocytes, macrophages and unengulfed melanin particles. The basal cells, some of which show mitotic figures, are 8 to 9 micra and their round or oval nuclei contain one or two nucleoli. The





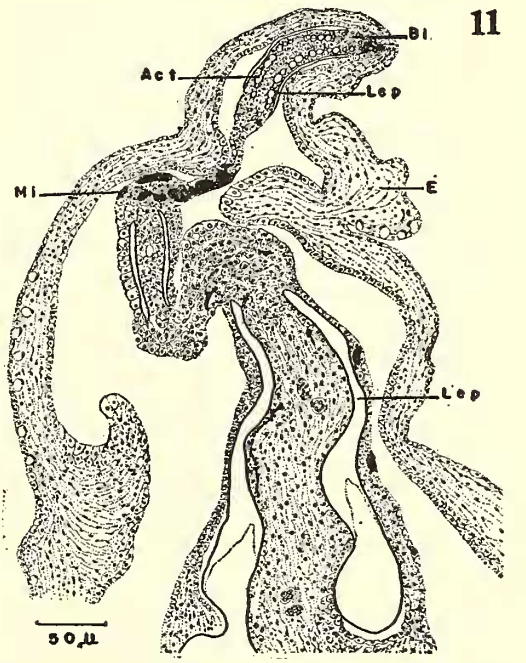
TEXT-FIG. 9. Details from blastema of regenerating dorsal fin of a comet platyfish, after one week, refer to Text-fig. 2b. **Mc**—Melanocyte between the blastema cells. **Bl.c**—Blastema cells. **Co.f**—Collagenous fibers at the base of blastema. **Er**—Erythrocyte.

TEXT-FIG. 10. Regenerating epidermis in the blastema, see Text-fig. 2d. Note one blastema cell in mitotic division. **Mc**—Melanocyte. **Ba.c**—Basal cells. **Bl.c**—Blastema cell. **Pm**—Pigment mass.

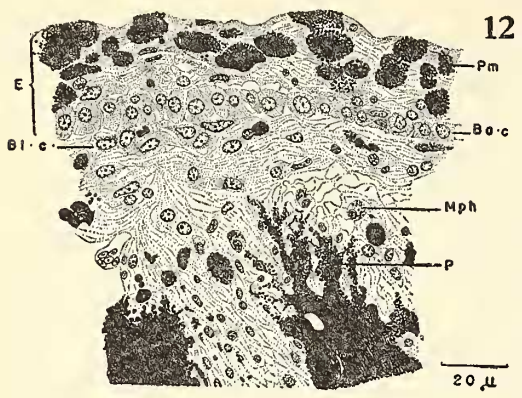
squamous cells are 5 to 7 micra and have elongated nuclei. The fibroblasts are round or oval, 5.5 to 7.5 micra, and have round or oval nuclei containing one or two nucleoli. The fibroblasts show some mitotic figures and are most active in the formation of the blastema proper, Plate I, Figs. 1, 2. The initial blastema contains no melanophores although its epidermis contains some free melanin and some macrophages with ingested pigment. Eventually the free melanin picked up by macrophages passes through the epidermis in the manner described by Bösenberg (1938) and by Gordon & Lansing (1943). Following this, melanocytes and young micromelanophores appear in the base of the blastema. The melanocytes are 14 to 15 micra; they are oval or spindle-shaped, but later they become dendritic. In Text-fig. 9 and Plate I, Fig. 3, a melanocyte is shown between blastema cells and collagenous fibres at the base of the regenerating fin, and in Text-fig. 10 another is shown just under the basal cells of the epidermis; here one blastema cell is in a stage of mitotic division. During the first week, blood capillaries appear in the blastema. Micromelanophores continue to increase as they move in along the connective tissue of the regenerating fin.

With regard to the regeneration of the fin rays, within one to two weeks after amputation, macrophages accumulate about the stumps of the fin rays and phagocytize the lepidotrichial debris. Later, the distal blastema cells begin to form actinotrichia and then lepidotrichia in a manner described by Blanc (1949). At first the regenerating lepidotrichia are not in contact with the ray stumps. Within three weeks those which form below the epidermis approach each other along their convex surfaces to reconstitute a fin ray. The proximal tips of the regenerated fin rays approach the distal tips of the old ray stumps and the distance between them is filled with additional skeletal elements from the blastema cells.

In Text-fig. 11, a diagonal cross-section of a dorsal fin 23 days after amputation, the lepidotrichia of two successive fin rays are shown. The epidermis of the fin is hyperplastic; the blastema



TEXT-FIG. 11. A diagonal section through a regenerated dorsal fin of a comet platyfish 23 days after amputation, see Text-fig. 2d. **Act**—Actinotrichia. **Bl** – Blastema. **E** – Epidermis. **Lep** – Lepidotrichia. **Mi**—Micromelanophore.



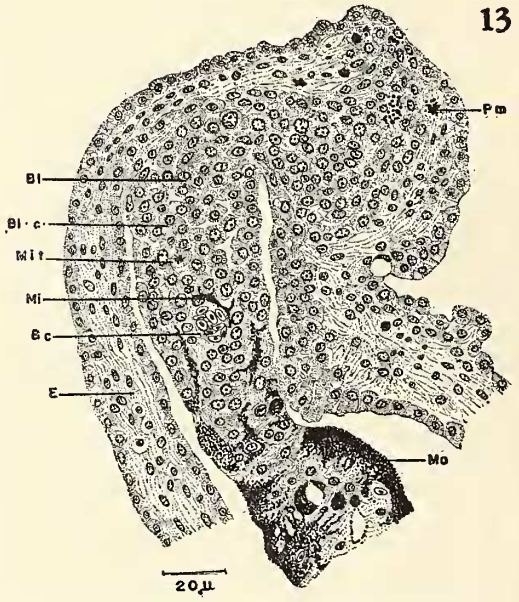
TEXT-FIG. 12. A section through a regenerating dorsal fin of a platyfish-swordtail hybrid four days following amputation of the fin, which had been in a state of melanosis, see Text-fig. 4c. **Ba.c**—Basal cell. **Bl.c**—Blastema cell. **E**—Epidermis. **Mph**—Macrophage. **P**—Processes of macromelanophore. **Pm**—Pigment mass in the epidermis.

cells are present in the distal part. The paired lepidotrichia of the old fin rays are located at the base of the fin. In this figure some micromelanophores are shown in the connective tissues around the fin rays. Within two to three months the regenerating fin reaches its previous dimension and then ceases to grow; the epidermal cells are no longer hyperplastic, the mucous and sensory cells reappear. Within four to five months the former pigmentation is restored in the regenerated fin.

From histological observations of the regeneration process, it appears that the most active growth takes place along the distal margin of the blastema, for it is there that mitotic figures are most frequently found.

Hybrids with melanosis of the dorsal fin reacted in a manner similar to the previous group following amputation, except for differences in pigment cell development. Within 12 to 48 hours following amputation of a melanotic dorsal fin, melanophore debris, free melanin, pigment-containing macrophages and collagenous fibres are present in the wound, Plate I, Fig. 4. Within three to four days the wound is covered by an epidermis and basal cells are restored. Macrophages and intercellular pigment masses are still present but they are eventually eliminated, Plate I, Fig. 5. The blastema cells accumulate under the basal cells of the epidermis, Text-fig. 12. This figure also shows some processes of macrophages that have entered the area of regeneration from below. Within six to seven days the blastema, which is covered by a hyperplastic epidermis, is formed, Text-fig. 13. Mucous cells in the epidermis are now present. Some blastema cells show mitotic divisions. Blood capillaries have developed. New micromelanophores are located in the blastema and macromelanophores are present in the base of the fin. Most of the macrophages and free pigment masses that were in the epidermis have been eliminated.

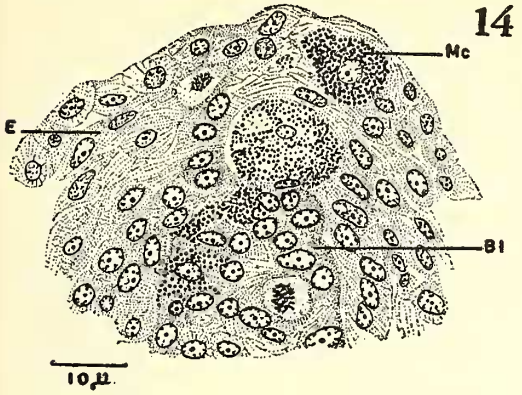
Melanocytes, the precursors of micro- and macromelanophores, are present between the blastema cells and in the epidermis, Text-figs. 14 and 15. They measure 11 to 22 micra and are round, oval cells, or spindle-shaped; later they may develop dendritic processes. It can not be said whether melanocytes come from the tissue under the epidermis or from the epidermis itself. Sometimes macromelanophores are located in the epidermis of the regenerated fin, Text-fig. 16. All of the macromelanophores apparently do not originate from melanocytes because macromelanophores that had been in the underlying tissues of the removed fin migrate into the regenerate, Text-figs. 4 and 17. As regeneration proceeds, the macromelanophores accumulate in



TEXT-FIG. 13. Cross-section of a blastema formed within six days in a similar fish, see Text-fig. 4. **Bc**—Blood capillary. **Bl**—Blastema. **Bl.c**—Blastema cell. **E**—Hyperplastic epidermis. **Ma**—Macromelanophore. **Mi**—Micromelanophore. **Mit**—Mitotic division in blastema cell. **Pm**—Pigment mass.

the connective tissue, where their pseudopodial processes anastomose and they recreate a state of melanosis in the regenerated fin, Text-fig. 18.

Two melanoblasts, 14 micra, each with fine melanin in granules, were found around a blood capillary of a one-month-old regenerated fin,

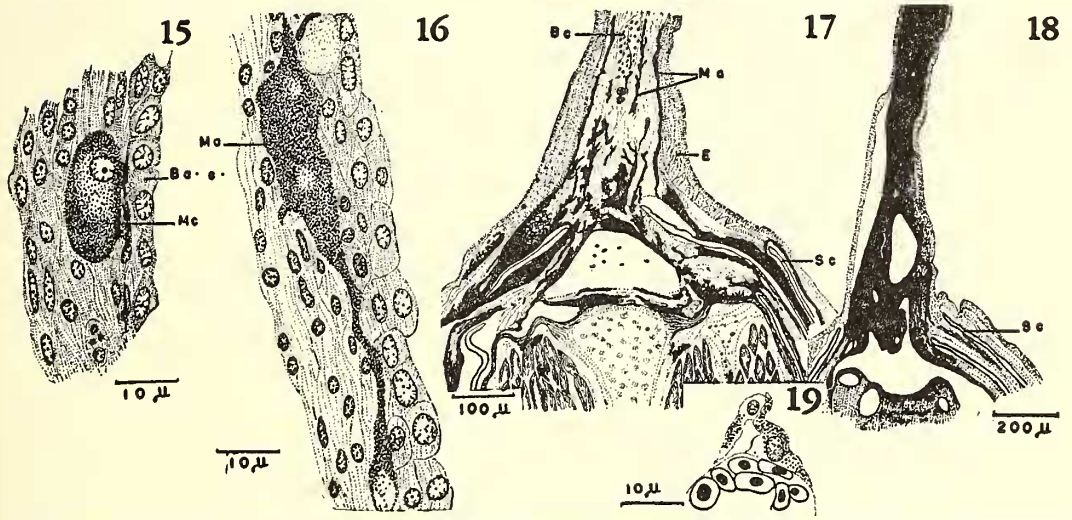


Text-fig. 14. Details of melanocytes in the hyperplastic epidermis in the blastema of similar fin, see Text-fig. 4c. **Mc**—Melanocyte. **E**—Epidermal cell. **Bl**—Blastema cell.

Text-fig. 19. This suggests that new pigment cells in the blastema may develop *in situ*.

Fin rays may regenerate in the presence of melanosis, but in a few of these regenerating fins all the rays did not redevelop. Lepidotrichia first reform in the distal part of the fin, while pigment and epidermal cells generally reappear in the proximal region. Scales that have been destroyed at the base of the fin may also regenerate. Within two to three months the regenerated fin attains its former height, but the degree of its pigmentation is different. The developmental rate of melanosis in the regenerating fin depends upon the age of the animal and upon the degree of melanosis that exists on the body ventral to the amputated fin.

In hybrids that have melanotic melanomas in their dorsal fins, the regeneration process is similar, in the earlier stages, to that in hybrids whose dorsal fins show strong melanosis. Within 24 hours after amputation of the fin, the regenerating epidermis covers the wound surface, Text-fig. 20 and Plate I, Fig. 6. From these figures it may be seen that beneath the amputated fin, in the body proper, connective and muscle tissues had been replaced by tumor cells. The regenerating epidermis is hyperplastic and the cells are vacuolated. There are many macrophages and pigment masses. A bleached section of the fin is shown in Plate II, Fig. 1; the paired lepidotrichia appear darker here than in un-



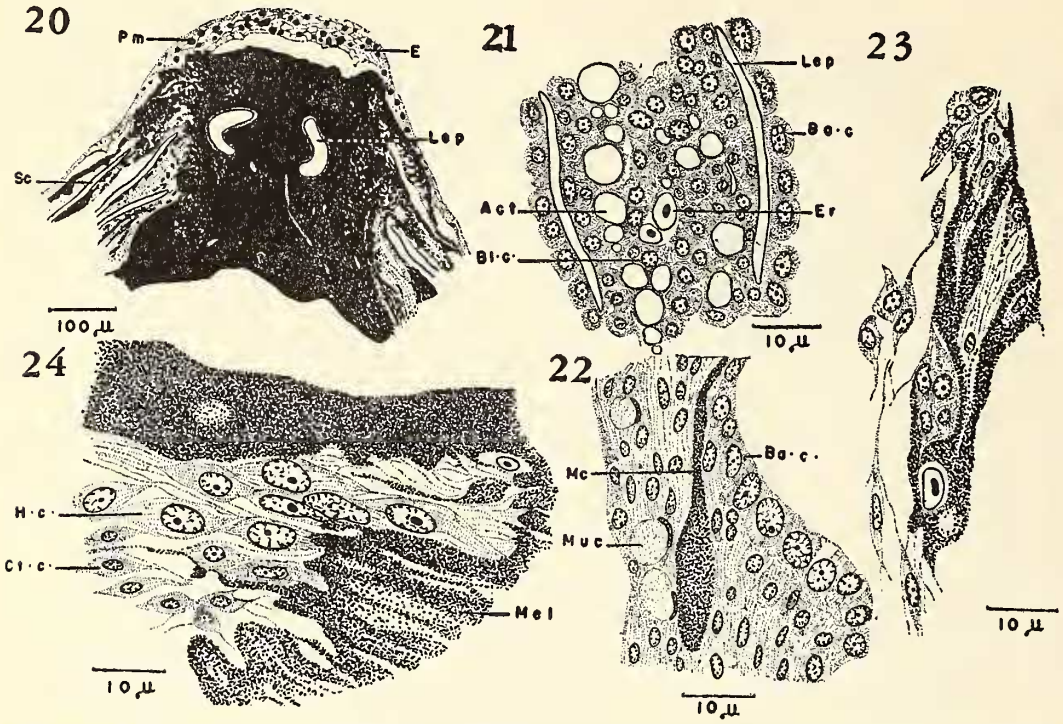
Text-fig. 15. Details of melanocytes in the hyperplastic epidermis in the blastema of similar fin, see Text-fig. 4c. **Mc**—Melanocyte. **Ba.c**—Basal cells of the epidermis.

Text-fig. 16. Macromelanophore in the regenerating epidermis of a blastema of a similar fish a month later, see Text-fig. 4f. **Ma**—Macromelanophore.

Text-fig. 17. Cross-section of similar fish showing part of the regenerated dorsal fin within one month following amputation and part of dorsal region of body. Macromelanophores have migrated into the regenerated fin from the region of melanosis below dorsal fin, see Text-fig. 4f. **Bc**—Blood capillaries. **E**—Epidermis. **Ma**—Macromelanophore. **Sc**—Scale.

Text-fig. 18. Cross-section through the regenerated dorsal fin after 6 months, see Text-figs. 4f, 4g. **Sc**—Scale.

Text-fig. 19. Two melanoblasts around a blood vessel of a one-month-old regenerated dorsal fin, see Text-fig. 4f.



TEXT-FIG. 20. Cross-section through the regenerated epidermis of an amputated dorsal fin after 24 hours. The dorsal fin had a melanoma which extended into the region below the fin, see Text-figs. 6, 6a, 6b. **E**—Epidermis. **Lep**—Lepidotrichia. **Pm**—Pigment mass. **Sc**—Scale.

TEXT-FIG. 21. Distal region of a 13-day-old blastema of a melanomatous fish, see Text-figs. 5b, 5c. **Act**—Actinotrichia. **Lep**—Lepidotrichia. **Ba.c**—Basal cells. **Bl.c**—Blastema cells. **Er**—Erythrocyte.

TEXT-FIG. 22. Hyperplastic epidermis in a similar fish after 45 days, see Text-fig. 5e. **Mc**—Melanocyte. **Ba.c**—Basal cells. **Muc**—Mucous cell.

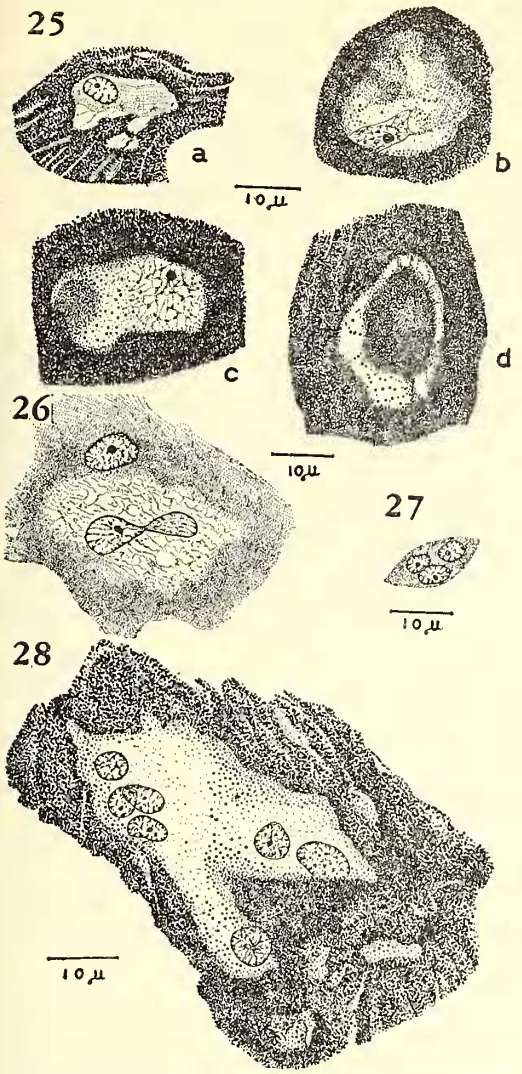
TEXT-FIG. 23. Macromelanophore surrounding a blood capillary in the connective tissue of a 13-day-old regenerated dorsal fin, see Text-fig. 5c.

TEXT-FIG. 24. Basal region of a regenerated dorsal fin, 13 days after amputation of a melanoma. The tumor cells' path of invasion of the regenerated fin follows the connective tissue, see also Text-fig. 6c. **H.c**—Hypertrophic fibroblasts. **Ct.c**—Fibroblasts, some are hypertrophic. **Mel**—Melanomatous tissue.

bleached sections, Plate I, Fig. 6. Within six to seven days the basal cells of the new epidermis appear over the wound's surface and the blastema is formed. The blastema cells, as is usual, originate from fibroblasts. In cases where the connective tissue beneath the amputated fin has been destroyed by tumor tissue, the blastema cells migrate from the nearest undestroyed connective tissues. Within 10 to 15 days actinotrichia appear, followed by the formation of paired lepidotricha, Text-fig. 21. During their regeneration some abnormalities occur, for example, sometimes three fin rays may regenerate to replace two.

Melanocytes and micromelanophores first appear in the blastema. Later, within two to three weeks, macromelanophores either migrate into the blastema from the region immediately below or they are reformed by melanocytes *in situ*. A melanocyte in the hyperplastic epidermis of a 2.5 months' old regenerated fin is shown in Text-fig. 22; Text-fig. 23 represents a macromelanophore around a capillary in the connective tissue.

After the condition of melanosis has returned in the regenerated fin, the hyperplastic growth of macromelanophores continues, the cells dividing amitotically. They become the principal tumor cells, replacing the connective tissue in the regenerated fin. The early fibroblasts become hypertrophic, Text-fig. 24; they measure 22 micra, are spindle-shaped, and their oval or round nuclei measure 8 to 10 micra. The extended pseudopodial processes of the macromelanophores first surround the fibroblasts, Text-



TEXT-FIG. 25. Pigmented hypertrophic fibroblasts at the base of a 13-day-old regenerated dorsal fin, see Text-figs. 6c and 24. **A**—Hypertrophic fibroblast surrounded by the pigmented processes of macromelanophores. **B**—Pigmented hypertrophic fibroblast, its melanin acquired from adjacent pigment-producing cells, macromelanophores. **C**—Pigmented hypertrophic fibroblast with nucleus in a state of karyolysis. **D**—Pigmented hypertrophic fibroblast with central mass of pigment granules.

TEXT-FIG. 26. Bleached section showing pigmented hypertrophic fibroblasts in amitotic division. The nucleus on the right is that of a macromelanophore which surrounds the secondary pigmented fibroblast, see also Text-fig. 6d.

TEXT-FIG. 27. Multinucleated spindle-shaped cell in the connective tissue of the regenerated dorsal fin, shown in Text-figs. 24 and 6c.

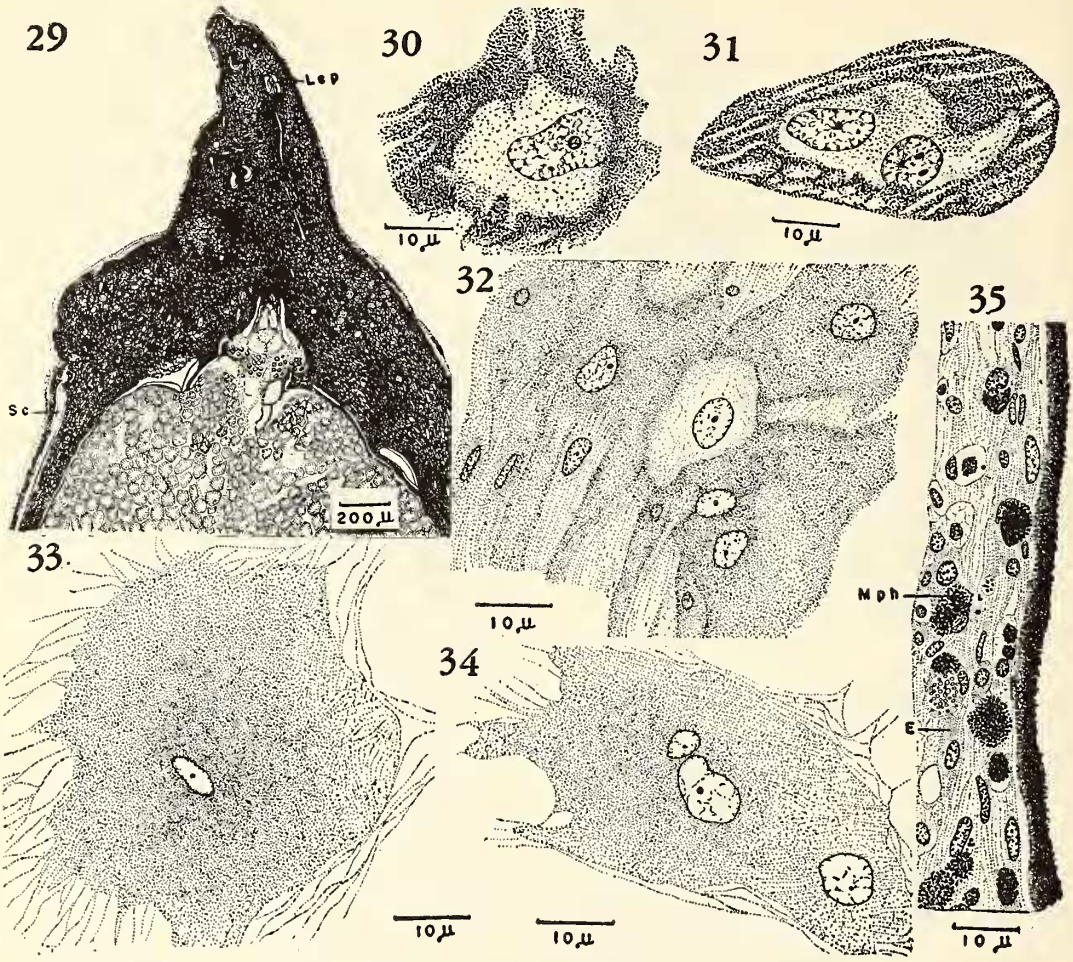
TEXT-FIG. 28. Giant cell with 7 nuclei from regenerated melanoma, see Text-fig. 6f.

fig. 25a. The fibroblasts obtain their melanin pigment by these contacts, Text-fig. 25b. The fibroblasts, which may be round, oval or polymorphic, increase to 25 to 35 micra and their nuclei measure 10 to 15 micra. Their nuclei divide amitotically with the result that in some cells two or more nuclei may be formed; this is illustrated in Text-fig. 26 which shows a bleached preparation. The nucleus at the periphery of the fibroblast shown in this figure also represents part of the macromelanophore which surrounds it. The fibroblasts which appear during the early stages of melanoma formation in the regenerate may be called "pigmented hypertrophic fibroblasts." The nuclei of some of these cells may disappear by the process of karyolysis, Text-fig. 25c. Melanin granules may reach the center of the fibroblasts and become concentrated there. Between this central mass of melanin in the pigmented hypertrophic fibroblasts and the surrounding macromelanophores, a nonpigmented ring may appear, Text-fig. 25d. The pigmented hypertrophic fibroblasts do not produce their contained melanin, but acquire it from adjacent pigment-producing cells. Some pigmented hypertrophic fibroblasts that developed in a melanoma are shown from a bleached section in Plate II, Fig. 2.

In the melanoma where pigmented hypertrophic fibroblasts develop, certain relatively small and polynucleate cells, measuring 18 to 19 micra, are occasionally found, Text-fig. 27. The cytoplasm of these rare cells stains more intensely with hemalum-eosin than the other cells in the regenerating tissues. These multinucleate cells possibly originate from the hypertrophic fibroblasts. In addition, some large polymorphous and multinucleate cells, which are about 500 micra in size and are called "giant cells," appear in the tumor tissue. One giant cell with seven nuclei is shown in Text-fig. 28. Probably these large cells are also derived from fibroblasts.

The connective tissue in the regenerated fin forms the fibrillar stroma of the developing melanoma. The connective tissue cells are relatively small, being 10 to 15 micra, and have nuclei 3 to 8 micra. Like fibroblasts, they may become pigmented during the hyperplastic growth of the macromelanophores. It is the hyperplasia of the tumor cells described above that causes a thickening of the fin which may even become nodular.

Macromelanophores are the principal tumor cells of the melanomas that redevelop in the regenerating dorsal fins. They measure 300 to 470 micra, their nuclei are 15 to 20 micra; they possess fine or lobulated dendritic processes. In the tumor tissue, they are found as individual cells or in complex aggregations forming syn-



TEXT-FIG. 29. Cross-section of regenerated melanoma that developed after 2.5 months in the dorsal fin in hybrid shown in Text-figs. 6, 6f. **Lep**—Lepidotrichia. **Sc**—Scale.

TEXT-FIG. 30. Pigmented hypertrophic fibroblast with one nucleus from melanoma shown in Text-fig. 29.

TEXT-FIG. 31. Pigmented hypertrophic fibroblast with two nuclei from melanoma shown in Text-fig. 29.

TEXT-FIG. 32. Bleached section of the dorsal fin melanoma shown in Text-figs. 6f and 29. Several macromelanophores form a syncytium within a fibrillar connective tissue stroma. A pigmented hypertrophic fibroblast is shown in the central area.

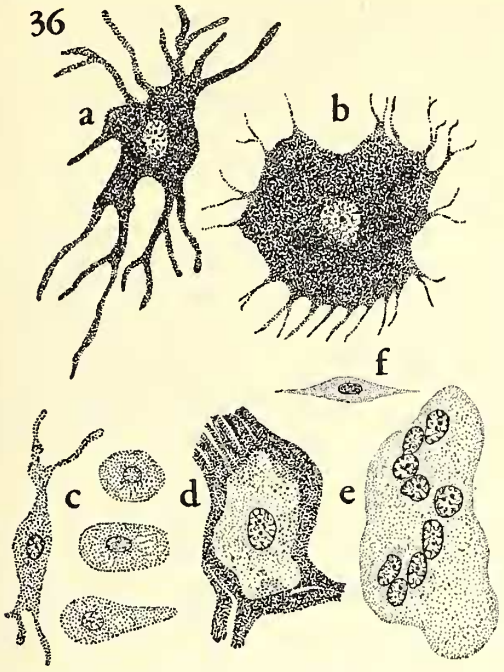
TEXT-FIG. 33. Bleached section showing an isolated macromelanophore from a melanomatous dorsal fin shown in Text-fig. 5f.

TEXT-FIG. 34. Bleached section showing an isolated macromelanophore with three nuclei from the melanoma shown in Text-fig. 5f.

TEXT-FIG. 35. Epidermis of a regenerated melanomatous dorsal fin, see Text-figs. 5e, 5f. **E**—Epidermis. **Mph**—Macrophage.

cytial masses in which cell boundaries can not be determined, Plate II, Figs. 3 and 4. In a bleached section of the melanoma, hypertrophic pigmented fibroblasts, connective tissue stroma cells and syncytial macromelanophores may be seen, Plate II, Fig. 5, The fibrillar structure of the cytoplasm of the macromelanophores may be seen clearly in this photomicrograph.

Two types of recurrent melanomas may be distinguished with regard to their origin and rapidity of growth. The fast-growing type is mainly composed of macromelanophores that have migrated into the reformed tumor from melanomatous tissues in the stump of the original dorsal fin and in the body just below, as shown in Text-figs. 6 and 29 and Plate II, Fig. 6.



TEXT-FIG. 36. Principal cells of the recurrent melanomas in the dorsal fins of platyfish-swordtail hybrids following amputation: **a** and **b**—Macromelanophores. **c**—Four melanocytes. **d**—Pigmented hyperplastic fibroblast. **e**—Giant cell. **f**—Stroma cell.

The tumor tissue in the regenerated fin, as well as in the basal region of the fin, have the same cellular elements and melanotic appearance. The processes of the macromelanophores lie parallel to each other or form swirls, Plate II, Fig. 4. Their cell boundaries are indeterminate, as indicated in the study of bleached sections. Most of these cells have migrated from their previous position in the basal parts of the removed fin and body, Plate III, Fig. 1. Other macromelanophores, arising from melanocytes, also participate in recreating the new tumor in the regenerated fin, but since they form a syncytial mass with the macromelanophores that migrate into the regenerating fin, it is difficult to distinguish between them. In this type of melanoma, pigmented hypertrophic fibroblasts, giant cells and connective tissue stroma cells are found. Two pigmented hypertrophic fibroblasts, one of which contains two nuclei, are shown in Text-figs. 30 and 31; Text-fig. 32, drawn from a bleached preparation, shows a pigmented hypertrophic fibroblast with several macromelanophores in a fibrillar stroma of connective tissue cells.

The more slowly-growing melanomas are those that require approximately ten months to redevelop *in situ*. These develop in hybrid fish exhibiting melanomas that were originally restricted to the dorsal fin, Text-fig. 5. They consist of isolated polymorphic macromelanophores which originate *in situ* from melanocytes in the regenerated fin, Plate III, Fig. 2. One hybrid developed a nodular melanoma in the posterior part of the regenerate, Text-fig. 5. Cross-sections of this tumor, Plate III, Figs. 2 and 3, show that it is not completely pigmented. The almost round or oval macromelanophores with fine processes are imbedded in a fibrillar and less pigmented connective tissue stroma. This may be seen in a partially bleached section of this tumor, Plate III, Fig. 4. Macromelanophores containing one or more nuclei are observed in other bleached sections, Text-figs. 33 and 34. The position of the lepidotrichia determines a radial arrangement of the fine processes of the macromelanophores in parts of the melanoma, as shown in Plate III, Figs. 4 and 5.

In both types of recurrent melanoma in the regenerated dorsal fins, certain tumor cells reveal a degenerative process in action, as indicated by pyknosis, karyorrhexis or karyolysis of their nuclei. Blood capillaries are well distributed throughout the melanoma and granulocytes and free erythrocytes are also present. The recurrent melanoma is usually covered by a thin epidermis, but in some instances the epidermal cells may be hyperplastic and may contain some macrophages and melanin masses, as shown in Text-fig. 35. The skeletal elements usually resist infiltration but they, too, may be destroyed by the tumor cells.

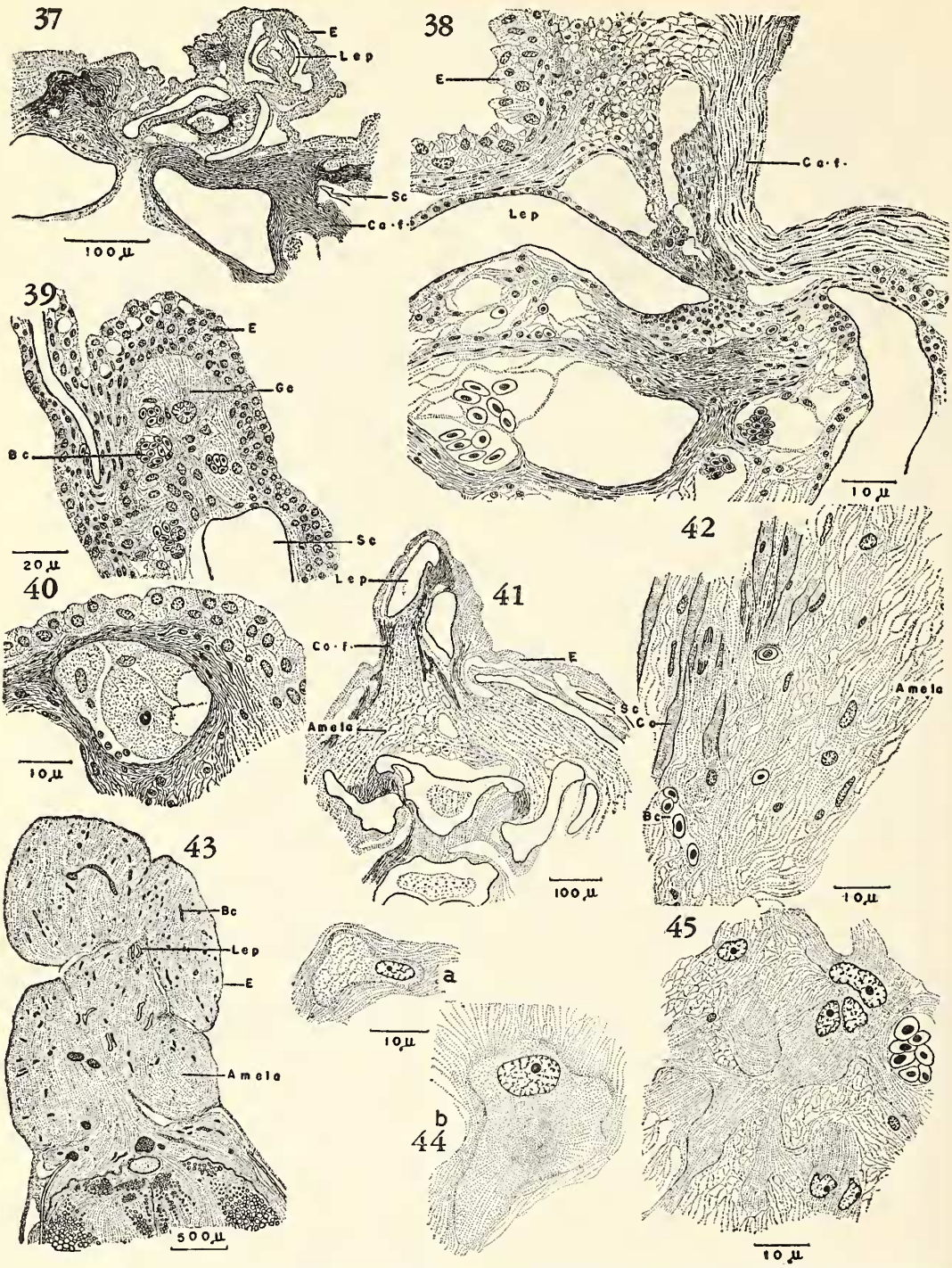
Thus, the first step in the recreation of melanoma in a regenerated dorsal fin is the development of a melanosis which is characterized by the proliferation and accumulation of macromelanophores. The macromelanophores then undergo progressive hyperplasia inducing, in turn, the hypertrophic growth of fibroblasts and giant cells. The macromelanophores invest these and other non-pigment-producing cells with melanin particles.

The most important pigmented cellular elements observed in the regenerating melanoma are as follows and are represented in Text-fig. 36:

a. *Melanocytes:* These are pigment-producing cells of 11 to 22 micra, with round or oval nuclei of 3 to 5 micra. They are usually oval or spindle-shaped, but may have wide lobulated processes. Melanocytes are capable of transforming into micro- and macromelanophores.

b. *Melanophores:* These are derived from melanocytes and are of two kinds:

Macromelanophores are large polymorphic cells measuring 300 to 470 micra, with round,



TEXT-FIG. 37. Cross-section of an amelanotic melanoma in the dorsal fin of a hybrid that reformed within 20 days after amputation, see Text-fig. 7c. **E**—Hyperplastic epidermis. **Lep**—Lepidotrichial element. **Co.f**—Collagenous fibers. **Sc**—Scale.

TEXT-FIG. 38. Section of the amelanotic melanoma shown in Text-fig. 37, under higher magnification.

TEXT-FIG. 39. Section of unamputated amelanotic melanoma tissue within a scale pocket at the base of the regenerated fin, see Text-fig. 7c. **E**—Epidermis. **Bc**—Blood capillary. **Gc**—Giant cells. **Sc**—Scale.

oval or polymorphic nuclei that are 15 to 20 micra; they contain one to two nucleoli and a loose network of chromatin. They have fine or lobulated dendritic processes. These are the cells that initiate the development of melanomas in platyfish-swordtail hybrids.

Micromelanophores are smaller, about 100 micra.

c. *Pigmented hypertrophic fibroblasts:* These cells have no dendritic processes. They are round, oval or polymorphic and measure 25 to 35 micra; their round or oval nuclei are 10 to 15 micra. They acquire their melanin from adjacent macromelanophores.

d. *Giant cells:* These are large polymorphic and multinucleated cells of about 500 micra. Their nuclei, which are round, oval or occasionally polymorphic, and are 5 to 10 micra in size, have a loose network of chromatin with one or two nucleoli. The giant cells may develop dendritic processes rarely; they also acquire pigment granules from true pigment cells.

e. *Pigmented connective tissue stroma cells:* These are spindle-shaped cells of 10 to 15 micra. Their oval or round nuclei measure 3 to 8 micra and contain dense granular chromatin. They, too, may acquire their small number of melanin particles from adjacent pigment-producing cells.

The regeneration process and recurrence of amelanotic melanoma in the dorsal fins of albino hybrids are essentially similar to these processes in dark hybrids with typical black melanomas. However, the cellular details may be more easily seen in sections of amelanotic melanomas and compared directly with bleached sections of ordinary melanoma.

The epidermis of a 20-day-old regenerated fin that had had an amelanotic melanoma is hyperplastic and many collagenous fibres are present, Text-figs. 37 and 38. The connective tissue and capillaries may be seen, as well as tumor cells, which remain below the amputated fin, in scale pockets and under the epidermis between the collagenous fibres, Text-figs. 39 and 40. The manner of infiltration by tumor cells into the connective tissue of a regenerated fin of the hybrid shown in Text-fig. 7 may be seen in Text-fig. 41. Some connective tissues and collagenous fibres which had formed in the regenerated fin are redestroyed and the fibroblasts are hypertrophic, Text-fig. 42. The nodular melanoma that developed in three months in the albino hybrid, shown in Text-fig. 8, when sectioned revealed the following details. The epidermis covering the overgrowth is almost normal but the vascularity of the amelanotic tumors is apparently greater than in the typical melanoma, Text-fig. 43 and Plate III, Fig. 6. Only three irregularly aligned dorsal fin rays are present in the tumor, the others being destroyed, Text-fig. 43. The connective tissue between the rays is replaced by the tumor cells, Plate III, Fig. 7. The trunk musculature at the base of the regenerated fin is separated from the melanoma by a layer of collagenous fibres, Text-fig. 43. In some areas this layer is destroyed by amelanotic melanoma cells which then infiltrate the musculature, Plate IV, Fig. 1.

The amelanotic melanomas in regenerated fins have a sarcomatous appearance histologically. The principal tumor cells are the amelanotic melanocytes and amelanotic macromelanophores. Some of these cells migrate into the regenerated fins from tumor cells below the fins, others are formed *in situ*. The colorless melanocytes and macromelanophores form syncytial masses in which cell boundaries are indeterminate. Their processes are sometimes parallel to each other; some form swirls, as

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TEXT-FIG. 40. Tumor cells surrounded by collagenous fibers at the base of a regenerated amelanotic melanoma, refer to Text-fig. 37.

TEXT-FIG. 41. Cross-section of a regenerated amelanotic melanoma in the dorsal fin after 3 months, shown in Text-fig. 7f. **Amela**—Amelanotic melanoma cells of the body invading the regenerating fin. **Co.f**—Collagenous fibers. **E**—Epidermis. **Lep**—Lepidotrichia. **Sc**—Scale.

TEXT-FIG. 42. Same as Text-fig. 41 but under higher power of magnification. **Amela**—Amelanotic melanoma cell from below the dorsal fin now in fin. **Co.f**—Collagenous fibers partly destroyed by tumor cells. **Bc**—Blood capillary.

TEXT-FIG. 43. Cross-section of amelanotic melanoma that developed in the dorsal fin of an albino hybrid within three months following amputation, see Text-fig. 8f. **Amela**—Amelanotic melanoma. **Bc**—Blood capillary. **E**—Epidermis. **Lep**—Lepidotrichial element.

TEXT-FIG. 44. Two types of tumor cells in amelanotic melanoma, refer to Text-fig. 8f. **a**—Cell with homogeneous cytoplasm, homologous to the pigmented hypertrophic fibroblast found in melanotic melanomas. **b**—Cell with radial fibrillar cytoplasm.

TEXT-FIG. 45. Giant cell with three nuclei in the amelanotic melanoma shown in Text-fig. 43.

shown in Plate III, Fig. 7, and Plate IV, Figs. 1 to 3. The cytoplasm of the macromelanophores has a fibrillar appearance. The melanocytes show mitotic figures, whereas the macromelanophores show amitotic ones. The cytoplasm of the non-pigmented hypertrophic fibroblasts in amelanotic melanomas is homogeneous and stains more lightly with hemalum-eosin than the other tumor cells, Text-fig. 44a. The non-pigmented giant cells have lobulated nuclei; some may be multinucleate, their nuclei dividing amitotically, Text-fig. 45, Plate IV, Fig. 4. Certain polymorphic cells (30 to 50 micra in size) in the amelanotic tumor have their counterparts in the pigmented hypertrophic fibroblasts of the typical melanoma. These polymorphic cells have fibrillar structures that radiate from a center (which can not be seen in the black melanoma), Text-fig. 44b and Plate IV, Fig. 5. Ermin (1946) described these pigmented hypertrophic fibroblasts as "sekundär" pigment cells which have one or more nuclei that measure 9 to 10 micra. Only the distal surface of the amelanotic melanoma was necrotic. Various types of cell degeneration, such as pyknosis and karyorrhexis, were observed. Sections of amelanotic melanoma, when compared with bleached sections of melanotic ones, reveal the fact that both types of tumor have almost the same fibrillar structure. Compare, for example, Plate II, Fig. 4, with Plate IV, Figs. 4 to 5. The lacunae described by Breider (1938) in gray melanomas on the bodies of "black albinos" and by Levine (1948) in amelanotic melanomas on the body proper—both of which are essentially the same—were not seen in the dorsal fin melanomas.

Finally, the amelanotic melanomas have some coarse granulocytes "in a discharging state" which are like those Catton (1951) described in various normal fishes and Aronowitz, Nigrelli & Gordon (1951) found in a spontaneous epithelioma in the platyfish *Xiphophorus variatus.*

Discussion

For studies of regeneration of the basic tissues of the dorsal fins and their pigmentation following amputation, certain fishes were chosen from many genetic stocks with a view of tracing the histories of two kinds of pigment cells, the micro- and macromelanophores. At first, fishes with micromelanophore patterns were studied; of these there were three kinds: (1) Wild type, *St* + +; (2) Wild type with a comet pattern, *St Co* +; (3) Wagtail, *St Co E,* see Table 1.

It should be noted that the micromelanophore pigmentation of the dorsal fin of the first two types is similar because the comet pattern is restricted to the caudal fin. In both dorsal and caudal fins of the wagtail, however, the number of these small pigment cells is much greater. Following amputation of the dorsal fins in all three types, the reformation of the fins and pigmentation requires about one month.

The sources of micromelanophores in the growing blastema and in the regenerated dorsal fin are: (1) from pre-existing melanophores on the base of the amputated fin and on the dorsal ridge of the body, just below the dorsal fin; and (2) from melanoblasts (or melanocytes) which come in with other cells to reestablish the dorsal fin, these pigment cells transforming *in situ* into melanophores.

This interpretation of the sources of melanophores in regenerated fins of fishes is essentially the same as those suggested by other observers, specially Bösenberg (1938), Goodrich & Nichols (1931), Wunder & Schimke (1935), Grimm (1949), Wunder (1951), Goodrich, Hine & Reynolds (1950), Goodrich & Bresinger (1953) and Goodrich, Mazullo & Bronson (1954), based on work on various species of freshwater and marine fishes. Bösenberg suggested that the melanophores were not migratory but were carried along with other cells into the regenerating fins. Goodrich, *et al* (1954), however, declared that some melanophores may enter as propigment cells and differentiate later; they are usually first observed as lightly pigmented cells having the migratory or ameboid form. Marcus & Gordon (1954) traced the movements of melanocytes in melanoma transplants and found that some melanocytes, after they transformed into melanophores, ceased moving.

The neural crest origin of pigment cells in lampreys and fishes was first suggested by Borcea (1909) and has been supported by Weidenreich (1912), Lopashov (1944), Newth (1951) and Orton (1953). But there may be other, as yet indefinite, sources of these cells, according to Oppenheimer (1950) and Goodrich (1950).

In the second series of experiments, the history of the restoration of the macromelanophore pattern in dorsal fins was studied in two genetic strains of platyfish: (4) Comet with spotted-dorsal, *St Co* + *Sd* and (5) Wagtail with spotted-dorsal, *St Co E Sd.* These were produced by intermating platyfish from two different geographical populations. The result in the next generation of this intermating, as shown by Gordon (1951a), is an increase in the intensity of macromelanophore pigmentation which reaches a point of atypical growth, that is, a low degree of melanosis. The spotted-dorsal fish with the comet and wagtail patterns were essentially similar in this respect. Somewhat similar also were the platyfish-swordtail

hybrids with various degrees of melanosis, item 7 of Table 1, *Sd*.

The restoration of pigmentation after amputation of the dorsal fin in *Sd* fishes depended primarily upon the state of original melanosis. For example, if the original melanosis was relatively light, then the regenerated fin was less pigmented. This confirms preliminary studies of this problem made by Goldsmith, Gordon & Nigrelli (1947). They found that the melanotic dorsal fins of five-month-old unoperated control platyfish-swordtail hybrids had more macromelanophores than the regenerated fins of 11-month-old sibling hybrids. They suggested that the difference might lie in the fact that the tissues comprising the regenerated fins are chronologically younger than those in the controls. In the present observations, if the dorsal fin originally had a strong melanosis, regeneration of pigmentation was more rapid and reached almost equal intensity after an 8½ month period. Younger hybrids had the capacity to develop the original state of melanosis more rapidly than older fish. This is interesting because Scott (1907) discovered that in *Fundulus heteroclitus* the regeneration rate of normal fins is greater in younger than in older fishes which, he believed, is in line with the theory that regeneration is a growth phenomenon.

In the reformation of the state of melanosis in the regenerated fins, one source of the pigmented cells, including the specific melanosis-producing macromelanophores, was through the migration of large melanophores from their position below the dorsal fin. Silber (1951) also found that macromelanophores entered the dorsal fin blastema of platyfish-swordtail hybrids from "pigment cell depots" located at the base of the fin. We have found a second source of macromelanophores in the regenerated fin, namely, that they are formed *in situ* from melanocytes. Recently Marcus & Gordon (1954) have also found that some melanocytes present in a transplanted melanoma transform into macromelanophores in host tissues.

After amputation, the reformation of a melanoma or an amelanotic melanoma in the dorsal fin of *Sd* hybrids (list in item 8, Table 1) depends upon the degree of tumor involvement not only of the original dorsal fin but of the body just below that fin. If the tumor development in the body below the fin is pronounced, the regenerated melanoma or amelanotic melanoma often exceeds the size of the amputated tumor, sometimes almost by three times. If there is little tumor involvement of the body below the fin, the reformed dorsal fin melanoma usually does not exceed that of the original tumor.

It is an interesting fact that the progressive growth of a melanoma may be halted temporarily by amputation of most of its tissues. Immediately after the operation, apparently there is sufficient normal tissue available in the stump of the dorsal fin to recreate the whole fin. Subsequently, the tumorous tissue that has not been removed grows, invades and destroys the newly regenerated tissues.

In the process of the reformation of the melanoma following amputation, the same sequences of tumor development were found as in the formation of the original melanoma. In hybrid fishes, the development of spontaneous and of regenerated melanomas is preceded by a premelanomatous state of melanosis in specific areas. In fishes carrying the sex-linked, dominant gene, *Sd,* the dorsal fin is the site of the melanosis, a condition brought about by the rapidly proliferating macromelanophores.

Gordon (1951a) pointed out that in pure platyfish, macromelanophores appear in the dorsal fin in response to the presence of the *Sd* gene only after three to five months, whereas in inter-racial hybrid platyfish with the *Sd* gene, these large pigment cells may appear in two weeks. In platyfish-swordtail, inter-specific hybrids, the macromelanophores in the *Sd* fish appear still earlier, some on the day of birth. Gordon (1948, 1950b) suggested that the rate of macromelanophore proliferation is accelerated in proportion to the strength and frequencies of other genes that modify pigment cell growth.

The atypical growth of these large pigment cells leads to a state of melanosis which may be destructive to adjacent normal tissues. The latter may be destroyed and replaced by them (Gordon & Smith, 1938). In the melanosis produced in platyfish-swordtail hybrids, the concentration of macromelanophores is so great that practically no other types of pigment cells can be distinguished. If the melanosis appears in the dorsal fin, as it does in *Sd* fishes, the fin may be destroyed at various levels, but there is no swelling of tissues. Gradually this phase changes and there appears a noticeable swelling. When sections are cut through the swollen areas and studied histologically, they reveal a significant change in cellular components. The outstanding feature of the new growth, as Reed & Gordon (1931), Gordon & Smith (1938) and Grand, Gordon & Cameron (1941) (by tissue cultures) have pointed out, is the preponderance of melanocytes and the relatively small number of macromelanophores.

The importance of understanding the transitional steps, from the appearance of macromelanophores in a genetically susceptible an-

imal, through their hyperplastic growth to the formation of a melanosis and, finally, to the development of a definitive melanoma, has been appreciated in former studies (Gordon, 1951b). But until the present work on regeneration and on transplantation (Marcus & Gordon, 1954), the cellular elements involved and their relationships to each other could not be properly evaluated.

There are two categories of pigment-carrying cells in the melanoma. One group contains those cells which not only carry melanin granules but are capable of synthesizing melanin pigment. This group includes melanocytes and melanophores, both large (macro.) and small (micro.). Pigment cells of the second group do not synthesize the few or many melanic granules that they carry. This group includes pigmented hypertrophic fibroblasts, pigmented giant cells, pigmented connective tissue stroma cells and pigmented macrophages (melanophages). These secondary pigment cells acquire their pigment by contact with cells of the first category through various processes.

In the transition from the state of melanosis to that of melanoma, the macromelanophores in their atypical growth form a dense syncytial mass in which their dendritic processes anastomose. Sometimes the melanophore processes are parallel, sometimes they form swirls. The cell membranes are indeterminate. The fibroblasts in contact with and in response to the progressive atypical growth of the macromelanophores, become hypertrophic and pigmented, possibly by cytocrine activity on the part of the dendritic, pigment-forming melanophores. Although we utilize Masson's (1948) concept of cytocrine activities, our use of the term *melanophore* is not the same as his; Gordon (1953) pointed out that many human pathologists have used the term *melanophore* to denominate what biologists call *macrophage*. The primary pigment cells, melanocytes and melanophores, have the property of liberating some of their melanin by clasmatosis (Grand, Gordon & Cameron, 1948). Melanin particles so released may be picked up by adjacent cells in the tumor, such as the fibroblasts, giant cells and other connective tissue cells. The pigmented hypertrophic fibroblasts may divide amitotically. These cells may lose their nuclei by the process of karyolysis. In some areas of the melanoma the fibroblasts form large oval bodies in which the acquired melanin is both peripheral and central. Similar pigmented bodies have been seen by Breider (1938, 1939, a, b), Ermin (1946) and Levine (1948). In another variation of the dorsal fin melanoma which externally appeared nodular, we have found macromelanophores of various configurations and with fine dendritic processes within a dense fibrillar connective tissue stroma.

The amelanotic melanomas, listed as 9 in Table 1, do not differ fundamentally from the typical melanomas except, of course, in the amount of melanin contained in the various cells. The details presented by Levine (1942) for amelanotic melanomas on the bodies of platyfish-swordtail hybrids have also been found to hold for those of the dorsal fin, except that the latter have a more prominant fibrillar network.

A comparison of the progressive stages in the regeneration of teleost fins with their normal development—based on the observations of many authors from Ryder (1885) and Harrison (1893, 1895) to Okado (1943) and Blanc (1949), and on the present studies—reveals that the regeneration process is essentially a repetition of the normal ontogenetic process. This similarity also holds for the restoration of the normal pigmentation patterns of the fins. Moreover, results obtained through the amputation of abnormal fins in a state of melanosis or with melanoma, show that here, too, the fundamental repetitive ontogenetic processes are evident.

It is well known that in the normal development of the teleost fin, temperature and other exogenous factors influence the nature of the growth process. Higher temperatures during certain critical development stages, for example, result generally in a smaller number of fin rays in the adult, and lower temperatures produce a higher count of rays, according to Hubbs (1922), Gabriel (1944), Täning (1952) and others. Recently Buser-Lahaye (1953) suggested that external influences (such as temperature and light) are not applied directly in the regeneration processes but are mediated through the endocrine glands among which the thyroid has a special role. In addition, although the part that nerve cells have been shown to play in regeneration in the amphibia has not been evaluated in fishes, it is probable that these cells are equally influential in teleosts. Indeed, the failure of some of the fin rays to regenerate in certain fishes may possibly be attributed to this factor.

Melanomas and other pigmented tumors in man and the normal pigmentation of the human body have been variously interpreted by pathologists with regard to cellular components and their embryological origin. This is evident by reading the more recent statements of Willis (1948), Masson (1951), Itô (1951), Becker (1948, 1953), Raven (1953) and Allen & Spitz (1953, 1954).

The subject is too involved for review here, but it seems to us that no discussion of pigment cells is complete without reference to Dawson's (1925) remarkable studies on human melanomas. We have found his well illustrated analysis of the progressive growth of human cutaneous melanomas most instructive, because he not only described the disease in its final, definitely pathological phase, but also he traced its ontological development from its earliest, apparently innocuous state. Studied by this dynamic method, the progressive history of human cutaneous melanoma shows striking parallels to those of fish. In comparing these histories it must be remembered that Dawson's term *melanophore* is equivalent to the biologists' *macrophage*. No true melanophores are found in mammals; melanophores are specialized effector cells characteristic of fishes, amphibians and reptiles. One of the important findings from studies of regeneration and transplantation of fish melanomas is that the true melanophore is related directly to the melanocyte and melanoblast. Thus it may be re-emphasized that the fundamental cell type in the melanomas of all vertebrate animals is the melanocyte.

Summary

1. The variously pigmented dorsal fins of fifty-three young and adult platyfish *(Xiphophorus maculatus)* and platyfish-swordtail hybrids *(X. maculatus-X. helleri)* were amputated. Their regenerated fins and pigmentary patterns were studied histologically.

2. It was possible to compare the results of amputation and regeneration of normally pigmented dorsal fins with those that were either in a state of melanosis or exhibited melanomas. Among the latter, it was also possible to compare the regeneration process in those that had typical black melanomas with those that had amelanotic melanomas.

3. All of the amputated dorsal fins regenerated in about two to three months, with essentially the same pigmentary pattern they showed originally. In some of the fishes with melanomas, regeneration was abnormal but it was not necessarily impeded by the simultaneous growth of a melanoma.

4. The restoration of melanosis in the regenerated dorsal fins was faster in young hybrids than in mature ones. The state of melanosis was incomplete in the regenerated fins of those fish in which the melanosis was confined to the fins alone. Melanosis was more complete in the regenerated fins of those fish that had melanosis in the fin and on the body below the dorsal fin as well.

5. The reformation of a melanoma in regenerated dorsal fins was more rapid in hybrids that originally showed a melanoma both in the dorsal fin and on the body ventral to the fin than in hybrids that had a melanoma in the fin alone.

6. The regeneration process and the recurrence of amelanotic melanomas in the amputated dorsal fins of albino hybrid fish were essentially similar to those in hybrids with typically black melanomas. The progressive growth of the melanomas was halted but only temporarily by amputation.

7. The same sequences were found in the reformation of the remissive melanomas that were observed in the development of the original melanomas.

8. After the dorsal fin was amputated, squamous cells of the epithelium moving from both sides of the wound grew over its surface and reformed the epidermis. Basal cells of the adjacent tissues moved under the squamous epithelial cell layer.

9. Melanocytes and micromelanophores appeared in the blastema before the macromelanophores. These are all true pigment cells that produce the melanin they carry.

10. Melanophores were derived from two sources: from normal pigmented areas immediately below the regenerating fin and from melanocytes that develop *in situ* in the blastema.

11. Some of the pigmented hypertrophic fibroblasts in the melanoma acquired their pigment from contact with macromelanophores either through the process of clasmatosis, cytocrine activity or both. This is also true of the pigmented connective tissue cells of the stroma and of the giant cells.

12. The regenerated amelanotic melanoma has a sarcomatous appearance histologically, as does the original tumor. The principal cells of the amelanotic melanoma are the amelanotic melanocytes and amelanotic macromelanophores.

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EXPLANATION OF THE PLATES

Photomicrographs of sections stained either with hemalum-eosin or by Masson's method.

Plate I

Fig. 1. The regenerated epidermis five days following amputation of dorsal fin of a comet platyfish. The blastema cells are below the epidermis. (Corresponds to Text-fig. 2b, an earlier blastema stage). 240 ×.

Fig. 2. Bud formed of blastema cells pushes the regenerated epidermis upward. (Corresponds to Text-fig. 2b). 600 ×.

Fig. 3. A melanocyte (Mc) in a one-week-old regenerating dorsal fin. (Corresponds to Text-fig. 2b). 1000 ×.

Fig. 4. Epidermis four days following amputation of the dorsal fin that exhibited melanosis. The collagenous fibers below the epidermis and the epidermis cells surround free melanin masses. (Corresponds to Text-figs. 4b, c). 300 ×.

Fig. 5. The elimination of pigment particles through the epidermis of a nine-days-old regenerating dorsal fin of a platyfish-swordtail hybrid that had a melanosis. (Corresponds to Text-fig. 4d, at an earlier stage). 600 ×.

Fig. 6. The regenerating epidermis 24 hours following amputation of a dorsal fin of a hybrid that had a melanoma in the fin and in tissues below the fin. The remains of one fin ray may be seen within the melanoma tissue. (Corresponds to Text-fig. 6b at an earlier stage). 100 ×.

Plate II

Fig. 1. A bleached section through the regenerating dorsal fin shown in Pl. I, Fig. 6. 100 ×.

Fig. 2. Part of the melanoma in the ventral region of a regenerated dorsal fin of an *Sd* hybrid with melanoma. Numerous pigmented hypertrophic fibroblasts are present in the tumor. (Corresponds to Text-fig. 6e). 440 ×.

Fig. 3. Section showing parallel arrangement of dendritic processes of macromelanophores in the dorsal fin of an *Sd* hybrid with melanoma. (Corresponds to Text-fig. 6f). 440 ×.

Fig. 4. A bleached section of melanoma of the same specimen showing swirl-like arrangement of processes of macromelanophores. 440 ×.

Fig. 5. Pigmented hypertrophic fibroblasts and a syncytium of macromelanophore processes in a bleached section of a regenerated melanoma of an *Sd* hybrid that had a melanoma in its dorsal fin. (Corresponds to Text-fig. 6f). **Ma**—Macromelanophore. **P.h.f.** – Pigmented hypertrophic fibroblast. **S.c.**—Stroma cell. 1000 ×.

Fig. 6. Part of regenerated tumor after 2.5 months in an *Sd* hybrid that exhibited melanoma. (Corresponds to Text-fig. 6f). 600 ×.

Plate III

Fig. 1. Bleached section of the three-week regenerated dorsal fin of an *Sd* hybrid showing a macromelanophore that has migrated from the base of the fin. (Corresponds to Text-fig. 6d). 600 ×.

Fig. 2. Section of the nodular melanoma in the 10-month regenerated dorsal fin of an *Sd* hybrid showing macromelanophores developed in the tumor. (Corresponds to Text-fig. 5f). 600 ×.

Fig. 3. Nodular melanoma reformed in the posterior part of the same hybrid as shown in Fig. 2. (Corresponds to Text-fig. 5f). 50 ×.

Fig. 4. Same nodular melanoma shown in Fig. 3 under higher magnification showing radial arrangement of processes of macromelanophores. 100 ×.

Fig. 5. Part of a bleached section of the same nodular melanoma shown in Fig. 3. 600 ×.

Fig. 6. Section of amelanotic melanoma that had regenerated in the dorsal fin of an albino (*Sd i*) hybrid. Note the hyperplastic blood vessels. (Corresponds to Text-fig. 8f). 100 ×.

Fig. 7. Section of amelanotic melanoma of an albino hybrid showing the replacement of the connective tissue between the lepidotrichia by tumor cells. (Corresponds to Text-fig. 8f). 600 ×.

Plate IV

Fig. 1. Section of amelanotic melanoma that redeveloped in the regenerating dorsal fin of an albino (*Sd i*) hybrid, showing the penetration and destruction of collagenous fibers at the base of the fin. (Corresponds to Text-fig. 8f). 600 ×.

Fig. 2. Section of an amelanotic melanoma showing amelanotic macromelanophores in the tumor tissue. (Corresponds to Text-fig. 8f). 950 ×.

Fig. 3. Another part of the amelanotic melanoma showing fibrillar structures in the tumor tissue. 600 ×.

Fig. 4. Two giant cells in an amelanotic melanoma. (Corresponds to Text-fig. 8f). 950 ×.

Fig. 5. Amelanotic melanoma showing cell (A) with radiating fibrillar structures. (Corresponds to Text-fig. 8f). 440 ×.